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STUDIES OF SINGLE MUSCLE FIBRES

I. The All-or-None Principle

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The retrolingual membrane (membrana basihyoidea) of the frog contains striated muscle fibres that are scattered in such a fashion as to make it possible to work with intact single fibres. In most cases there is considerable microscopic space separating one fibre from another, and the whole preparation is one layer of fibres thick. This constitutes a very convenient object for the excitation of single fibres by the method that is outlined below.

This membrane containing the few striated fibres was described as long ago as 1890 by Ranvier. Very recently Fischl and Kahn (1928) have utilized this preparation by observing under the microscope the contractions of single fibres when the membrane was stimulated. By measuring with an ocular micrometer the extent of contraction of single fibres they observed graded responses to graded stimuli. Though the observations were made on single fibres, the entire membrane was being stimulated by metal electrodes and the possible influence of the other contracting elements on the one under observation could not be eliminated.

Using very small non-polarizable microelectrodes the writer was able to stimulate single fibres by the application of the electrodes directly on the fibre. It is possible under these conditions to make certain that only one fibre is responding and to study its behavior under excitation.

These microelectrodes, which have been described in detail elsewhere (Gelfan, 1927), consist of fine quartz capillaries drawn out to points of about 5μ in diameter at the tips. Allowing for the thickness of the walls of the capillary tubes, the actual opening at the tip, or electrode surface, is somewhat smaller. The capillary tubing is filled with agar dissolved in

[1] Donnelley Fellow in Physiology.

frog's Ringer's solution (for electrodes that are to be used on frog tissue) to the very tip. The other end of the capillary is sealed into a glass shank and covered inside the shank with the same agar. The rest of the shank is filled with Ringer's solution into which dips the Ag-AgCl coil, making it a non-polarizable system.

The membrane is excised essentially in the manner described by Fischl and Kahn (1928). During the dissection the membrane is continuously bathed by Ringer's. After the dissection it is flattened and spread on a glass cover slip by a camel's hair brush. The membrane is now covered by a strip of filter paper larger than the cover glass and soaked in Ringer's solution. The filter paper has a section cut out of its center, thus revealing that portion of the underlying membrane which contains the muscle fibres. The entire preparation is now placed over the moist chamber so that the exposed portion of the membrane is underneath facing the interior of the moist chamber while the cover glass is above. The moist chamber is mounted on a microscope and the tips of the electrodes are placed in position in the interior of the moist chamber by a micromanipulator. The tips of the electrodes, which are bent at a 90° angle, point vertically from the interior of the moist chamber to the muscle fibres.[2] The filter paper covering the membrane is continuously kept wet by the addition of Ringer's solution. The preparation can thus be kept alive without apparent injury for a number of hours if care is taken against drying.

Because of the thinness of the membrane it is possible to make observations with transmitted illumination; and with the microelectrodes coming up from underneath it is also possible to work with high magnification. With the micromanipulator the microelectrodes can be placed directly underneath any fibre. When the tips of the electrodes are touching the fibre they occupy only a part of the diameter of the fibre.

Single fibres were stimulated both by single break induction shocks and by tetanizing currents of short duration. The single fibres were repeatedly observed to give graded responses to changing stimuli for both the break shocks and the tetanizing current. The responses ranged from minute twitches to maximal contractions. With lower power of magnification, bringing many fibres into the field, whether one or more fibres are contracting with any given stimulus can be definitely ascertained. With higher power of magnification also, it can be observed that the response is localized in one fibre since the adjacent and immediately neighboring fibres that are in view do not contract. In the latter case, when the stimulated fibre is approaching maximal contraction as the stimulus intensity is increased, the immediately adjacent fibres may passively be pulled along. The difference in such instances between a passively moved and wrinkled fibre

[2] For diagram showing the relationship of the micro-electrodes moist chamber, and experimental object, see Gelfan, 1927.

and active contraction is unmistakable. With stimuli, however, that are just above threshold intensity, fibres that are as close to the responding one as to even touch it do not show any perceptible movement.

The measurement of the magnitude of the contractions with an ocular micrometer was found not satisfactory. This is especially true when single induction shocks were used. The response is too rapid to follow with the eye for accurate measurement. The mercury droplet method of Pratt and Eisenberger (1919) was therefore utilized for the graphic records that are here given.[3]

At first, attempts were made to use the membrane *in situ* and with circulation intact. With the membrane *in situ* and direct illumination, the visibility is not very good and the manipulation of the microelectrodes is difficult and uncertain. Though the graded responses of single fibres could be observed, it was not feasible to make photographic records under these conditions. The membrane was consequently excised and prepared in the manner outlined above. The exposed part of the membrane containing the muscle fibres was sprayed liberally with minute mercury droplets by an atomizer. The preparation is then submerged in Ringer's in a shallow glass dish and placed on the mechanical stage of the microscope. In this case, however, the exposed part of the membrane faces upward. This, of course, is necessary in order to have the mercury globule, which is illuminated from above, reflect the image up the microscope tube. Over the ocular is the photographic arrangement for recording the excursions of the mercury globule that is on the responding fibre. The detailed description of the apparatus of this photographic method is given in the paper by Pratt and Eisenberger (1919).

The microelectrode is moved into position by the micromanipulator. In this case the electrode, whose tip is bent at a 45° angle to make it convenient for orientation and visibility, is pointing down on the preparation. For these records the other microelectrode was used as an indifferent one and placed in the Ringer's solution bathing the membrane. A fibre is chosen that has the smallest mercury globule on it, the latter being preferable because it can be moved more easily by the contracting fibre. It is also necessary to choose one that lies directly on a fibre. The smaller contraction of a single fibre will not readily move the droplet unless the latter lies directly over it. Transmitted illumination is still used for the orientation of the field, and the electrode is gently placed directly on the surface of the chosen fibre. The mercury globule is then brilliantly illuminated from above, and as the excursions of the globule are being photographed, it is under constant observation throughout the experiment through a side tube. The constant observation while making the photo-

[3] It is with pleasure that I here acknowledge my gratitude to Doctor Pratt; with his coöperation the photographic records were made in his laboratory.

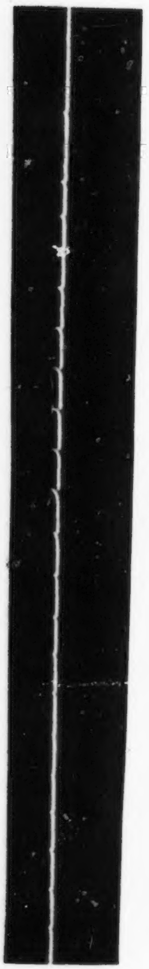

Fig. 1

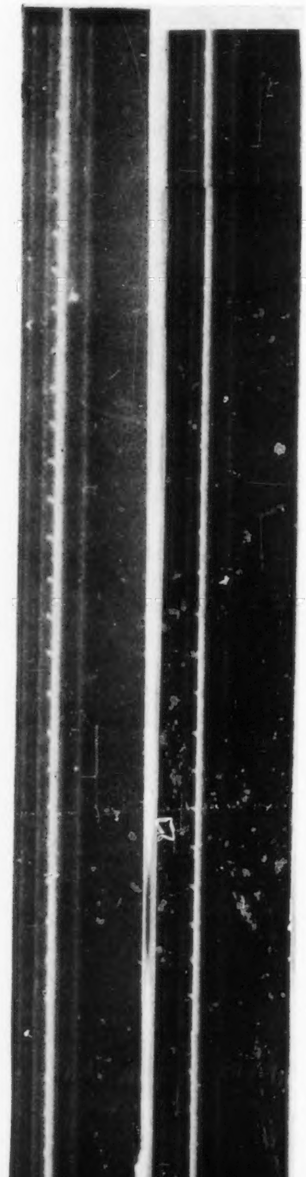

Fig. 2

Fig. 1. Photomicrographic record traced by an illuminated mercury globule on single fibre of excised retrolingual membrane (R. pipiens). Microscopic magnification, ×20; enlarged ×5; total, ×100. One micro-electrode applied over fibre recorded, the other in Ringer's solution bathing the membrane. Break induction shocks every 3 seconds; Cu amalgam-Hg contact on metronome interrupter. Stimuli graded by rheostat in primary. Record reads from right to left.

Fig. 2a and b. Continuous record, cut for reproduction; reads from right to left; experimental conditions are the same as in figure 1

graphic record is necessary in order to ascertain that only one fibre is responding.

Figure 1 is a record of a single fibre showing the excursions of a mercury globule that was lying directly over the fibre. The stimuli were graded by a rheostat in the primary. The increase and the decrease of the current intensity produced by a movement in the rheostat caused a corresponding gradation in the magnitude of the responses in the single fibre, as the record indicates. Figures 2a and b constitute a single continuous record (cut in two for reproduction) showing again the graded responses to graded stimuli. In this record the experiment was repeated.

Some of the smaller responses shown in figure 2 were produced by the same strength of stimulus. Wishing to reproduce the very minute twitches of the fibre, stimuli of the same intensity were repeated. The weakest and most minute responses that were still visible to the observer through the side tube were not sufficient to move the mercury droplet, at least move it to the extent that it could be recorded by the photographic plate. It is to be noted, however, that the minute excursions of the globule that are barely visible in the photograph, the total magnification of which is 100, do represent very small responses and a small fraction of the possible maximal contraction of the fibre.

The continuous record showing the repetition of the experiment indicates that it is not a phenomenon of fatigue. Furthermore Pratt (1917), and Pratt and Eisenberger (1919) found upon fatigue no change in the character of the response of sharply localized regions of skeletal muscle, only a rise in the threshold. Though the number of stimuli used, most of which were sub-maximal, were hardly sufficient to produce fatigue, it is possible that fatigue ensues sooner in this preparation since the membrane is excised and has no circulation. But figures 2a and b, and especially figure 1 at the very beginning of the record show the graded responses. It is not likely that at the very beginning of stimulation the fibres would be fatigued. Furthermore, it has already been pointed out that the graded response of a single fibre can also be observed in the membrane with intact circulation.

Pratt, in a paper elsewhere in this issue, raises the question of the possibility of interconductance among the fibres of this membrane. His histologic examination of the material shows apparent anastomosis in some cases. In his own experiments, nevertheless, he finds that the response does not spread from one fibre to another as one would expect if there existed true protoplasmic continuity between them.

If the musculature were truly compound, of the nature of a syncytium like the heart muscle, all the fibres of the membrane should contract, and only one type of response should be found in any given range of stimuli according to the all-or-none principle. A localized contraction in a syncytium

like the heart, however, would indicate either that the transmission was incomplete, or that the response may be graded, possibly both. In the normal and intact heart of Limulus, a direct mechanical or electrical stimulation, not involving the ganglion, always produces a contraction that is confined to the area stimulated (Carlson, 1908). The histological examinations (Meek, 1909) of the myocardium of Limulus, indicates that it is a syncytium like the vertebrate heart. According to Carlson (1908), the Limulus heart, unlike the vetebrate heart, does not under normal conditions conduct, but in pure isotonic sodium chloride solution the ganglion free Limulus heart does conduct. Carlson's suggestion of the possible mechanism whereby this change of physiological conduction is effected by the sodium chloride is relevant: "It is possible that the conduction is simply the result of the increased excitability of the muscle, so that the action current of one muscle strand or fibrilla is able to stimulate its neighbor." Such a mechanism of stimulus propagation by action currents might explain the all-or-none behavior of the normal vertebrate heart.

It is possible in the retrolingual membrane to mechanically stimulate what appears under the microscope to be a single fibre, to complete any maximal contractions by piercing the sarcolemma with the microelectrode (detailed account of this work will be reported in a future paper). The response does not spread to any of the remaining fibres, indicating that the musculature in this membrane is not a syncytium. There may often be seen, especially in stained preparations of this membrane, what appears to be the anastomosis of two fibres. Stimulation of such a compound fibre should also produce only one type of response if the all-or-none principle is valid for this musculature. If there is a membrane separating the two portions of the compound fibre, there should only be two steps. The records show a considerable number of gradations of responses and as far as could be judged under the microscope only one fibre was contracting.

With microelectrode stimulation, using threshold stimuli, twitches of the fibre have been repeatedly observed that were so minute as to be detected only with the higher power of magnification. These responses appear as if even in the single fibre they were localized. We have in this instance the possibility of partial transmission. It is interesting to point out that under certain conditions conduction with a decrement can be demonstrated in the iron wire model (Lillie, 1920). In the single fibre, however, with responses that are greater than the minute twitches, the transmission seems to be complete throughout the length of the fibre. This is most convincingly observed with tetanizing current. It is important to point out that the magnitude of the contractions of a single fibre, when stimulated by tetanizing current, increases with increase of duration of the stimulus.

The all-or-none principle has repeatedly been reported to hold in skeletal muscle[4], but in the musculature of the retrolingual membrane it seems to fail. The most obvious explanation that might be suggested is that the failure of the all-or-none principle is a characteristic only of this particular striped muscle and that it may still be valid for other striped muscles. The method of stimulation used in these experiments is yet to be tried on other types of striped muscles.

Keith Lucas (1905, p. 137) concluded that "If any continuous gradation of contraction of a single skeletal fibre can occur, it lies completely within a range of stimulus far smaller than that required to bring a whole muscle from rest to maximum activity." With the microelectrodes the stimulus intensity can be graded to a much finer degree than by the usual form of stimulation. Because of the minuteness of the tapering points of the microelectrode, the resistance of the latter is about one million ohms. A movement of the secondary coil or the rheostat in the primary circuit, because of the tremendous electrode resistance, produces a very small change of the stimulating current intensity. (A large resistance in the circuit would serve the same purpose for stimulation with coarse electrodes.) The fibres of the retrolingual membrane are very sensitive to minute changes of the intensity of the stimulus. If, with a sub-maximal stimulus producing a small twitch in the single fibre, the microelectrode, which was touching the fibre, is moved slightly so that a layer of Ringer's several μ thick now intervenes between the electrode and the fibre, this same fibre will not respond to the same stimulus that produced a twitch when the electrode was directly on it. Moving the electrode again into its former position will evoke a response. It is also necessary, in order to avoid the spreading of the stimulus to many fibres, that it should be localized to the extent that the microelectrodes touch only one fibre. The finer gradations of current intensity usually seem to be ineffective unless it is so sharply localized. It is possible, therefore, that the usual all-or-none response may be due to the more diffuse form of stimulation, and that very intimate and localized stimulation will give graded responses.

While observing the graded character of the minute twitches of a single responding fibre, one is often impressed with the apparent discontinuity of the responses of even the single fibre, as if the fibre itself were composed of contractile units. The photographic records, however, do not show discontinuities, or at least not distinctly so, in the graded responses of a single fibre. It is possible, however, that this method of recording is not fine enough to detect such discontinuities. If such sub-fibral independent contractile units did exist, we might still postulate an all-or-none behavior for them.

[4] Keith Lucas (1905), Symes and Veley (1911), Mines (1913), Pratt (1917), Pratt and Eisenberger (1919), Eisenberger (1917), Adrian (1922).

SUMMARY

1. A method is described by which single muscle fibres can be stimulated. The minuteness of the microelectrodes and intimacy of contact with fibre easily permit the localization of response to a single fibre.

2. In the musculature of the retrolingual membrane, graded responses of a single fibre are produced by graded stimuli, both by single induction shocks and tetanizing current. The magnitude of the response of a single fibre also increases with increase of duration of the tetanizing current.

3. The graded responses can be observed when the membrane is *in situ* with intact circulation as well as when it is excised. Other evidence is presented indicating that the graded responses are not caused by fatigue.

4. The possible reasons for the failure of the all-or-none principle in this case, when it has repeatedly been reported to be valid for other striped muscles, are discussed.

I am indebted to Professors Lillie, Carlson and Gerard for their valuable criticisms of this paper.

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ON THE GRADING MECHANISM OF MUSCLE

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The gradual approach of responses to maximal in voluntary muscle excited with progressively increasing electric shocks is in common experience difficult to demonstrate except with a uniform source of current and means of interrupting this with great precision. These conditions fulfilled, a series of increasing, narrowly spaced stimuli will yield a continuous gradient from minimal (the least observable response) to a value not affected by further strength of excitation.

Consider the conditions of this experiment early in the submaximal series as the muscle is made smaller and smaller. This may be accomplished by employing progressively smaller muscles, or by reducing the muscle by splitting, or by limiting the excited area through the use of progressively finer electrodes. Gradation is still present but is eventually interrupted, no matter how delicate the spacing of stimuli, by recurring discontinuities in the response gradient. Finally the curve of variation in size of response has been transformed from a smooth incline to a set of steps. Moreover the height of any step is sufficiently determinate to be regained at will, to be repeated on a descending series of stimuli and to resist the disturbing influence of slight departures from uniformity in the stimulating apparatus. The requirement of great nicety in delivering stimuli, characteristic of the grading of many fibers, becomes relaxed when few are involved, so that less attention need be given to clean contacts and breaks or evenly spaced movements of coil or rheostat. The mode of response seems determined, not by the precise intensity of the stimulus, but by an inherent property of some muscle unit.

This muscle unit, which Keith Lucas identified with the fiber, has become generally recognized as the element compounded in the adaptive gradations of muscular activity, similar in the all-or-none capacity of its discharge to the nerve fiber which excites it and insuring for any given set of determining conditions a determinate response.

Evidence from experiment is unequivocal that changes of stimulation intensity many in number, delivered to few muscle fibers, elicit changes of response few in number (Lucas, 1905). These changes are associated with

the abrupt action or elimination of actually observed fibers (Eisenberger, 1918). That the nervous system can bring about a similar stepping of effect is seen first in Lucas' (1909) classical experiment and more recently in reflex and tonic all-or-none reactions such as those secured by Porter and Hart (1923) and Porter (1929).

The tendency to read into the expression "all-or-none" the conception of an absolute, fixed degree of activity is responsible for the failure on the part of many to grasp the true significance of the principle. It should be obvious that protoplasmic activity can hardly attain uniformity except in an approximate sense and under experimental conditions. Embedded in an elastic matrix, through its intimate junction with neighboring cells[1] the muscle fiber if acting singly must, as Athanasiu (1927) insisted, be limited in its shortening by the mechanical resistance of its attachments. One is not justified, however, in deducing from this fact the slightest modification of the all-or-none law. Although noting the appearance which active fibers give of gliding between neighboring inactive fibers, Pratt and Eisenberger (1919) have explicitly drawn attention to the deformation of the contiguous tissue in all fiber activity. This mechanical limitation and modification of response by the resistance to deformation of attached structures is only one of the many environmental and intrinsic variables which, acting before or during contraction, may make the "all," whether measured in terms of space or of energy, a fluctuating function. To such influences, whether mechanical or physio-chemical, the response may bear a continuously dependent relation. To at least one influence, however, it appears to partake normally of the trigger-release type of action. This trigger influence is the stimulus, in the narrow sense of the term; and, since it does not follow through and limit the discharge which it releases, necessitates gradation by participation of an increasing or decreasing number of separate units, making response-magnitude a *discontinuous function of stimulus-magnitude* or, if stimuli hold a uniform intensity, of threshold value (Pratt, 1917). Under experimental conditions, where the manifold variables otherwise influencing response are in abeyance, the varying of stimulation strength reveals this discontinuity as a uniform "all" for any given step of response-increment. Such uniformity is not a universal criterion of the law, but merely the experimental test of its validity. It means that the disturbance of factors other than stimulus is eliminated in the interest of controlled observation by maintenance of the preparation in a balanced state.

The above statements reflect the theory of graded response as based on

[1] The junctions, however extensible, are sufficient to prevent the successful teasing apart of muscle fibers for experimental isolation; but the "working" attachments are terminal. For the mechanism of fractional contraction in relation to structural arrangement, together with cited literature, see the paper of Sybil Cooper (1929).

experiments thus far with the frog's sartorius muscle, using stimuli applied with electrodes fine enough to excite one or a few fibers only. Its validity, however, as an exclusive description of gradation must not go unquestioned, nor is a fundamental protoplasmic property to be too rigidly assumed in the all-or-none concept.

In the first place evidence is largely negative against the existence of a narrow realm of grading capacity overstepped when the fiber jumps from quiescence to its limit of response. This question, raised by Lucas (1919), is discussed at length by Pratt (1917) and by Pratt and Eisenberger (1918), who emphasize the importance of exploring the threshold region with thoroughness before assuming that an all-or-none effect is actually present. Under the conditions of their experiments, in which a pore electrode was used, the gradients of response were in all cases step-like; that is, no evidence of intrinsic grading was found.

Attention was drawn by Ranvier (1890) to that peculiar musculature in the frog, derived from the tongue, which invades in a thin, sparsely disposed layer the delicate membrane limiting dorsally the retrolingual lymph-sac (sinus basihyoideus). These fibers were not only described, but were submitted to electrical stimulation in a moist chamber devised by him for the purpose.

Recently a valuable detailed account of this membrane has been given by Fischl and Kahn (1927) who have, further, observed under the ocular micrometer the extent of movement of individual fibers, exciting the preparation diffusely by means of delicately graduated induction shocks. With the method employed the magnitude of response appeared as a function of stimulation strength, thus invalidating in these authors' opinion the all-or-nothing law for this type of muscle. They call attention to the fact that its fibers, derived from voluntary striated layers of intrinsic lingual muscle, must be regarded as unquestionably skeletal in fundamental properties, and incline therefore to a universal interpretation of the results.[2]

Certain noteworthy characteristics, however, distinguish this structure: *1.* The fibers, as is indeed not uncommon in tongue muscle, are distinctly—often profusely—branched. *2.* The branches in many cases anastomose, and *3*, are inserted distally into the areolar network of the tissue by means of strands of extraordinary delicacy. The musculature is thus suggestive of cardiac structure to the extent of having a virtually syncytial arrangement with apparent provision for interconductance and for direct traction on the tissue of which it forms a part. Ranvier, in fact, observing the similarity, accorded the membrane a function like that of the lymph-hearts —the branching and anastomosing fibers indeed suggesting a structural

[2] Fischl and Kahn give a comprehensive bibliography dealing with the all-or-none concept for muscle.

analogy. It will be found, however, that neither of these structures forms a fully conducting continuum. The areas of activity are strictly limited in both, and, in the case of the retrolingual membrane, the branches and commissures show a functional partitioning or dovetailing off of definite segments, especially at the junctions of branches with fiber trunks. Frequently a fiber resolves itself into a fasciculus of two or three strands, each functionally independent but in a common sheath.

Since Fischl and Kahn's method of stimulating was diffuse, in that the nerve when employed was excited macroscopically by wire electrodes, and the muscle by metallic plates on either side of the excised membrane, we must assume the possibility here of multiple-unit gradation. This in appearance would be continuous with the stimulus gradient, owing to the large number of units engaged in activity. Without graphic evidence these results must therefore remain indeterminate. It is certain that the tongue musculature has a structure peculiarly adapted for very fine gradation by units, owing to the multiplicity of functional segments involved in any fiber-complex. This profusion of available units makes possible a series of contractions under direct intensive stimulation, such as in figures 3 and 4 (hyoglossus), where the graded effect is perfect for the larger contractions but distinctly discontinuous for the smaller, which show the typical all-or-none relation so well brought out on the isolated fibers of the membrane (figs. 2, 5 and 6).

That this type of striated muscle can, however, be made to exhibit intrinsic grading is shown by Gelfan's work, reported elsewhere in this issue. Using the well separated fibers of the excised membrane and stimulating them individually by very fine micro-electrodes, Gelfan has unquestionably demonstrated contractions which are partial as regards the fiber, and graded with graded stimuli. Although the rôle of such gradation in the presence of nerve impulses of assumed standard intensity is problematical, the proof that the protoplasm of a type of striated muscle is capable of such gradation is of intense interest. Gelfan's conclusions are thus consistent with those of Fischl and Kahn and based on results less capable of other interpretation. Whether this gradation is compounded of definite units of effect within the fiber or is decremental in character, it will be of importance to determine.

Results by the graphic method on the retrolingual membrane. (With the collaboration of Marion A. Reid.) The experiments now described have utilized essentially the same method of stimulation and graphic record as that employed by Pratt and Eisenberger (1919). It has involved *a*, an examination of the excursions of single branches of the membrane fiber-complex in response to graded stimuli; *b*, an examination with graded stimuli of the underlying M. hyoglossus. The outcome of this study has proved consistent with that of work on the sartorius: rela-

tively diffuse excitation (as with an active electrode applied to the closely packed, multiple fibers of the hyoglossus) yielding graded contractions to graded stimuli; sharply localized excitation (as with the same electrode applied to a naturally isolated fiber-member of the membrane musculature) producing, within a wide range of stimulus intensities, responses of independent magnitude.

Fig. 1

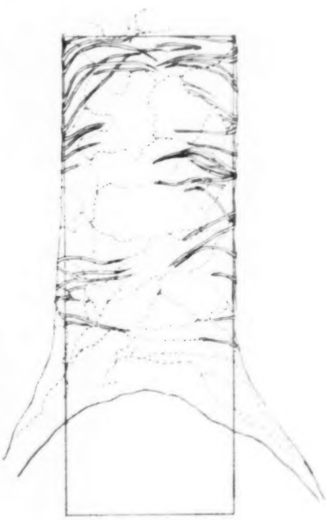

Fig. 2

Fig. 1. The slip of thin cover-glass, M, lies in the sinus basihyoideus beneath the membrane, insulating its musculature from that of the hyoglossus muscle which lies ventral to the sinus.

Fig. 2. Showing the rectangular piece of cover-glass in position under the membrane with muscle fibers and capillaries (dotted lines) as viewed by opaque illumination with the dissecting binocular. Only the main fibers are thus brought out. The finer branches and anastomoses are best seen by transmitted light in the excised membrane. This diagrammatic sketch is to emphasize the impressive isolation of fibers susceptible of stimulation.

Method. The cranium anterior to the eardrums is removed by transverse decapitation, leaving intact the mandible and entire floor of the mouth. The tongue is stretched horizontally forward and held by the weight of two artery clips. A transverse slit is made across the posterior margin of the *membrana basihyoidea* and an oblong fragment of coverglass, its corners and edges fire-polished, thrust through this opening in a forward

direction. This separates and electrically insulates the membrane (*M*, fig. 1, fig. 2) from the hyoglossus lying beneath. The membrane is now strewn with fine mercury droplets from an atomizer, the entire body is immersed in Ringer's fluid above the level of the membrane, and the preparation placed in the recording apparatus.

For stimulation, induction shocks are delivered through a no. 46 Calido-metal enameled wire (35μ in diameter), the strength of the shocks being under control of a sliding rheostat in the primary circuit in addition to that of the mechanism varying the position of the secondary coil. This active electrode is carried on the same stage which supports the preparation and is itself further actuated by a separate adjustment. The electrode may thus be brought to bear lightly over any muscle fiber observed through the demonstration ocular of the recording microscope. The indifferent electrode is formed by a metal sinker immersed in the solution covering the preparation. Shocks are delivered periodically by a Cu amalgam-Hg metronome-driven interrupter in the primary circuit.

Record is made by photographing on a slowly moving film the excursions 1, of a brilliantly illuminated mercury droplet resting over a stimulated fiber; 2, of a small spot of light automatically moved in the ordinate direction in accordance with the movement of the secondary coil (Pratt and Eisenberger, 1919).

The chief advantages of the above method are; continuance of circulation through the tissue, limitation of electrode contact to an area approximating the diameter of a muscle fiber, and graphic evidence of actual change

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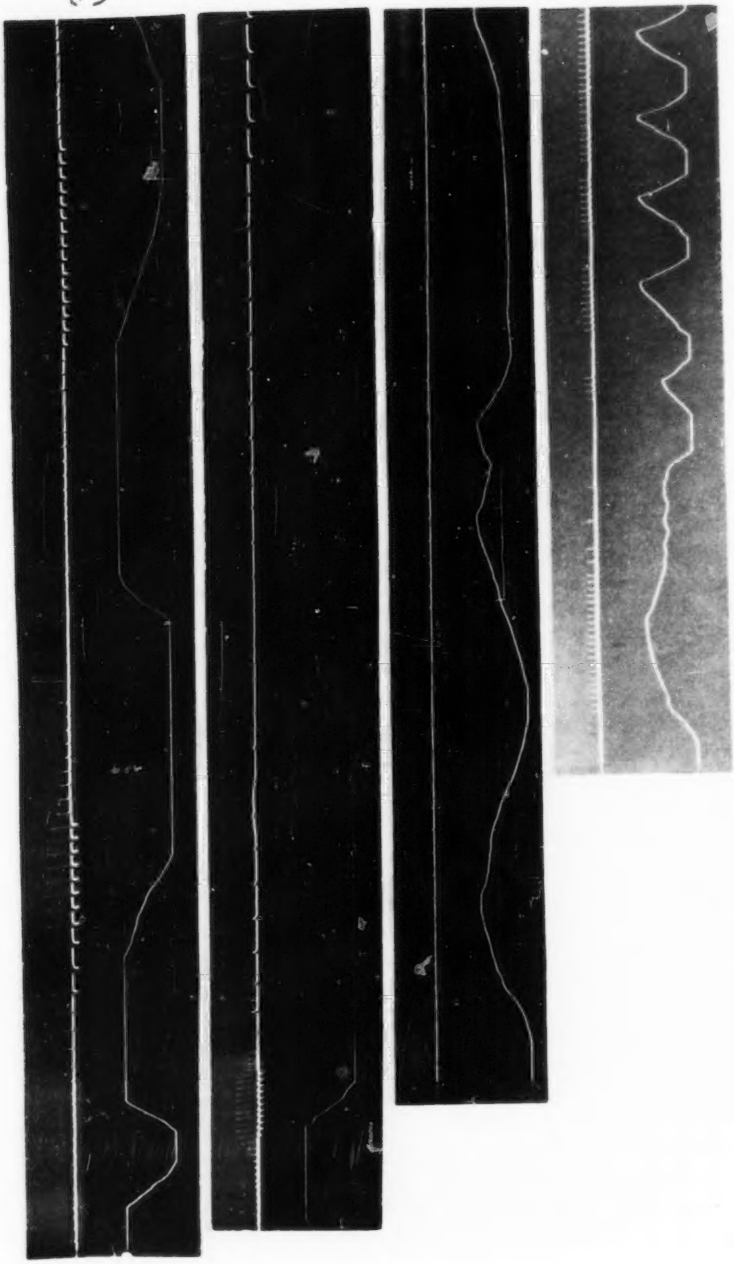
Fig. 3. Hyoglossus. Break shocks, 20 per minute. The lower curve records absolute movement of secondary coil. At the levels between these movements the primary current was also varied by a rheostat so as to increase the current after every increase of secondary intensity (upward) and to decrease it after every decrease (downward). The lower twitches show step-like gradation; the higher twitches, continuous gradation from the entrance of new fibers at each current increment. With the maximal twitches the entire field moved. Here a recoil is suggested by the extension of twitch tracings below the abscissa.

Fig. 4. Hyoglossus. The same preparation and stimulating conditions as in figure 3. The first graded series is with a more slowly moving film. Discontinuous grading is apparent only in the smaller contractions.

Fig. 5. Retrolingual membrane (membrana basihyoidea). Break shocks, 18 per minute, graded by movement of secondary coil only, the record of which appears in the lower curve. Only one degree of response appears, even with very fine grading near the threshold at the end of the tracing.

Fig. 6. Retrolingual membrane. Break shocks, 60 per minute. By slowly reducing the secondary current (first descent of lower curve) an attempt is made, by exploring the threshold, to reduce the minimal response. By increasing the primary current during the levels between the succeeding records of coil movements, a higher maximal is sought. Aside from slight gradients referable to treppe there appears no exception to the uniformity of contractions.

3 4 5 6



Figs. 3-6

in stimulation intensity accompanying the record of contraction. We regard the fine wire electrode for this kind of stimulation as superior to the pore type of liquid electrode, since the flexible wire remains in contact with the tissue at the same point during the excursion instead of permitting a sliding contact. A non-polarizable system is not required, and even with the relatively large size of the active electrode single trunk or branch fibers of the membrane may be singled out and made to contract independently of a commonly innervated group.

In the accompanying cuts of photographic records the original size of the contact print is preserved. This represents a real-image magnification of 20 diameters, produced by a Zeiss AA objective with special eyepiece. Smooth gradation is effected 1, by turning a lever actuating simultaneously a secondary coil and the signal spot (tracing the lower curve); 2, by turning the milled head of a rack-and-pinion rheostat adjustment, varying primary resistance. Details of the separate records are given in the legends.

The important discrepancies between our results and those of Fischl and Kahn on the one hand and of Gelfan on the other are to be referred to various possible factors.

Fischl and Kahn observe in a diffusely excited membrane the excursions made by an object in the field. We have repeated this method and do indeed find gradation, but of the sort to be expected in any musculature thus stimulated. Near the threshold, however, individual fibers may be seen contracting in a generally quiescent field; as already stated the preparation is peculiarly adapted for showing the variation in the number of active members or segments of fibers as the stimulus varies. Thus far gradation is apparently quantal—that is, composed of all-or-none steps. Reducing, now, the stimulus and varying it by means of a second and very delicately adjustable rheostat bridged across the primary terminals, we have eventually succeeded in reducing the contractions of a localized region of the fiber-complex to a flicker of one limited region which may itself show the all-or-none character.

Gelfan's work, in contrast with the above method, is done by actual or approximate contact of a region of high current density (micro-electrode) with the individual muscle fiber (trunk, branch, or commissure). One fiber alone is excited, and shows intrinsic grading. Since the membrane muscle lies in a naturally dissected state—its trunks and branches often isolated by spaces many times fiber-width—it lends itself readily to individual fiber stimulation by relatively gross means. For this reason we have been able to employ on the membrane, excised as in the above method, the same fine wire used in previous experiments. Thus we have been able not only to note the extreme ease with which all-or-none effects are obtained but, on particularly careful grading near the threshold, have been able to observe what are unquestionably submaximal twitches. In certain cases

these are governed by change of stimulus, as is undoubtedly the case in Gelfan's experiments. To this extent we have been able to confirm by a less favorable method the latter's results, which have since been further verified under more rigorous conditions.

These minute or partial contractions do not, however, appear on our records made from the tissue *in situ*. In the curves it will be seen that stimulus crosses threshold level repeatedly. By this method, apparently, no submaximal values are revealed. The evidence is negative. And at present we have no knowledge of whether the nerve in its control of muscle can do better than this, so far as bringing out a grading capacity for single responses is concerned. It would appear that it is not so much a special method of stimulation which is essential to a fractional response of the fiber (although apparently it can be shown most adequately by use of electrodes actually in contact with the fiber) but special attention to the most delicate grading in the threshold region under conditions still to be determined. The limits of the all-or-none effect are under investigation in the light of the new findings. There is much cumulative evidence in recent years from various sources that complete conduction is characteristic of the normal phasic response of the vertebrate skeletal fiber. The value of any data tending to harmonize this mode of behavior with graded or decremental processes is evident.

Even if intrinsic grading of the muscle fiber could be generally demonstrated only in the presence of fatigue, asphyxia or injury (such as must accompany the use of excised tissue)—conditions which Verworn (1913) regarded as changing the mode of response to one of gradation—the distinction set up, like the concept of fatigue itself, must be purely relative. If elicitable at all, grading should lie at least potential in all states of muscle. Granted the limitations of the somatic nervous system require the multiple unit (all-or-none) type of response for prompt and accurate grading of its effects, it is possible that this has been accomplished as an adaptation by the crowding of any grading capacity possessed by the individual fiber into a range too narrow to be readily revealed by the usual methods of excitation. Adopting this concept for the nerve, its grading (decremental?) capacity which may be evident in the lower forms (Jordan, 1929) can in the vertebrates be regarded as reduced to zero.

SUMMARY

1. The prevailing view of muscle fiber activity in relation to excitation regards size of response as a discontinuous function of strength of stimulus, though a continuous function as related to influences other than stimulus.

2. Recent evidence tending to modify the above concept denotes on the other hand an intrinsic capacity to respond in a graded manner to graded stimuli (Fischl and Kahn, Gelfan).

3. By the use of the mercury-droplet method of photographic recording, tracings are obtained showing the ease with which apparent gradation can be obtained, even by highly localized stimulation, from the frog's hyoglossus muscle, although similar stimulation applied to the naturally isolated fibers of the retrolingual membrane (from intrinsic tongue muscle) give an unquestionable all-or-none reaction.

4. It is concluded that these fibers, even if capable of intrinsic graded activity, share with the usual type of skeletal muscle the marked tendency to grade by units, the degree of activity of which is not determined by strength of stimulus.

5. In the presence of assumed intrinsic grading capacity the more apparent all-or-none effect may be regarded as an adaptation to the needs of the nervous system through intense narrowing of the range of gradation. Inability to demonstrate this range in a given type of muscle need not therefore place that muscle in a class fundamentally apart.

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PHYSIOLOGICAL VARIATIONS OF THE CARDIAC OUTPUT OF MAN

VII. The Effect of High Altitude on the Cardiac Output and Its Related Functions: an Account of Experiments Conducted on the Summit of Pike's Peak, Colorado

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The principal purpose of the present investigation was to determine the effects of low barometric pressures, as encountered in residence at high altitudes, on the cardiac output of man. It was also desired to ascertain the relationship, if any, of such manifestations as mountain sickness, acclimitization, etc., to the circulatory mechanism as measured by the pulse rate, blood pressure, cardiac output, and hemoglobin content. Several previous investigators have attempted the solution of this problem, but the results of these previous expeditions have been inconclusive. The Anglo-American Pike's Peak Expedition (Douglas, Haldane, Henderson, and Schneider, 1913) as a result of indirect measurements, concluded that the circulation rate as a whole was more rapid on Pike's Peak than at sea-level. Kuhn (1913) using the method of Plesch (1909), found an increase in the cardiac output, which ranged in 4 individuals from 3 to 28 per cent, at an altitude of 10,750 feet (3277 meters). The Anglo-American expedition to Peru (Barcroft, *et alii*, 1923) measured the cardiac output of two individuals by various methods based on the Fick principle. The authors conclude that "neither a rise nor a fall in the minute volume of 20 per cent was established at *Cerro*, whilst a change of a larger order was disproved."

Working conditions on Pike's Peak. The experiments recorded in the present paper were made on the summit of Pike's Peak, Colorado, during July and August, 1929. The general conditions on the summit of Pike's Peak have been so fully described in previous reports (Douglas, Haldane, Henderson, and Schneider, 1913; Henderson, 1914) that they need not be reiterated here except as regards such details as pertain specifically to the present work.

There are a number of factors which differentiate life at high altitudes from residence at lower levels. Thus apart from the lowered atmospheric pressure and consequently diminished oxygen tension of the inspired air, one must consider the altered solar radiation (as compared to sea-level),

the low humidity, wind velocities, low temperature, ionization of the atmosphere, etc. As regards the circulatory system of man, however, it is the effect of the low oxygen tension of the inspired air and the low temperatures which are the chief factors of importance in altering the cardiac output. It is the first of these factors in which we are most interested since it is the most inherent property associated with higher altitudes. Hence, in order that the measurements obtained at high altitudes may be comparable to those obtained at sea-level, it is essential that no factors occur which, independently of the low barometric pressure, might in themselves affect the measurements under consideration. This is particularly important in the case of such a labile function as the cardiac output which, as has been demonstrated in previous publications (Grollman, 1929b, c, d, e) may be influenced by such factors as cold, psychic disturbances, etc. All such disturbing factors were avoided in the present work and hence the results are considered as accurately representative of the effect of only one factor, namely, normal residence under reduced barometric pressure at high altitude.

TABLE 1

The results of daily measurements obtained on A. G., ♂, age 27; height 164 cm.; weight 74 kgm.; at Baltimore (sea-level) from May 23 to 29, 1929

PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
per minute	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
62	98/65	239	58	4.12
60	98/68	239	60	3.98
63	98/65	230	60	3.83
65	97/62	232	60	3.87
60	100/65	235	61	3.85
62	98/63	238	57	4.18
60	100/70	234	60	3.90

Living conditions on the summit of Pike's Peak were entirely normal and comparable to those at sea level. The chief subjects of the experiments, A. G. and L. C. G., did not lose weight as a result of their long sojourn on the mountain (contrary to the experience of most previous expeditions), the former, in fact, gaining 3 kilograms. Except for a rather mild attack of mountain sickness by L. C. G. during the first 36 hours after arrival on the Peak, the subjects were in perfect health throughout their stay on the mountain. The effect of extreme cold is a particularly troublesome factor at high altitudes. Fortunately, Pike's Peak during the first three weeks of the present study, was favored with an unprecedented warm and equable climate. The daily electrical storms, described by the Anglo-

American expedition, occurred only during the last week of the stay on the mountain, and, in general, the climate was comparable to conditions at Baltimore during the late autumn. Food, heat and other living conditions were otherwise like those described by Douglas, *et alii* (1913) and by Henderson (1914). Since the Anglo-American Expedition's visit to Pike's Peak in 1913, the summit of the Peak has been inproved by the construction of an automobile road affording additional communication with the base of the mountain and of an additional building in which the present experiments were conducted. The top of the Peak has also been cleared of its granite boulders thereby offering a circular space, several hundred meters in circumference with a negligible gradient. This latter improvement was particularly valuable for the study of exercise.

Methods employed. The determinations of the cardiac output recorded in this paper were made by the use of acetylene as described in

TABLE 2

The results of daily measurements obtained on L. C. G., ♀, age 30; height 158 cm.; weight 58 kgm.; at Baltimore (sea-level) from August 28 to September 3, 1929

PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
per minute	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
52	98/65	192	65	2.95
60	95/68	195	64	3.05
55	95/65	180	61	2.95
58	97/68	180	62	2.94
60	95/65	186	60	3.10
58	94/62	180	60	3.00
60	93/60	185	65	2.85

previous communications of this series. Precautions were taken to avoid all the factors which have been shown to affect the output of the heart. The largest series of determinations were made on two subjects, A. G. and L. C. G. These subjects had been under investigation for a period of over a year. As will be shown in a future communication, their cardiac outputs when taken under strictly basal conditions, in bed, immediately on arising in the morning after a good night's rest, is constant within the experimental error of the methods employed. Representative examples of this constancy are shown in tables 1 and 2 in which are recorded results obtained at sea level over a period of a week. Having thus demonstrated the constancy of the cardiac output of these subjects over long periods, one may draw conclusions from a series of experiments conducted at high altitudes under the same conditions.

In the method for determining the cardiac output utilized in the present

investigation, one determines the arterio-venous oxygen difference between the arterial and mixed venous bloods and calculates the actual cardiac output from this difference. The arterio-venous oxygen difference is obtained from a measure of the rates of absorption of a foreign gas (acetylene) and of oxygen, under the conditions of the experiment. The rate of absorption of acetylene is dependent solely on its solubility under the experimental conditions and on the rate of blood-flow through the lungs. The amount of oxygen taken up by the blood is dependent on its alveolar concentration, the rate of blood-flow, and on the oxygen content of the venous blood. In experiments conducted at sea level, the blood normally leaves the lungs saturated to the extent of 96 per cent of its oxygen capacity. As may be seen by reference to an oxygen dissociation curve, the oxygen tension of the alveolar air may vary from 80 to 110 millimeters without causing any great change in the oxygen saturation of the arterial blood. Hence in applying the Krogh-Lindhard principle for determining the cardiac output at sea-level, under normal conditions, it is not necessary that the average oxygen tension of the experimental gas-mixture which is rebreathed be exactly equal to the oxygen tension of the alveolar air before the experiment.

At high altitudes, however, the above considerations no longer apply. The alveolar oxygen tension at the summit of Pike's Peak allows only about 86 per cent saturation of the arterial blood. Any variation in the oxygen content of the alveolar air will markedly alter the amount of oxygen taken up by the blood. In order to obtain the normal resting arterio-venous oxygen difference it is, therefore, necessary that the average alveolar tension of oxygen during the experimental period (i.e., between the times of the collection of the samples for analysis) should be equal to the normal resting alveolar oxygen tension. One may easily calculate the amount of oxygen to be added to the mixture of gases rebreathed so as to approximate this desired concentration. In the present work duplicate determinations were always made and the mixtures were prepared so as to contain oxygen contents slightly higher and slightly lower, respectively, than the desired concentration. One could then interpolate between the results of the two determinations in order to obtain the correct value. It is also possible to correct for the deviation of the average alveolar oxygen content from the normal content by aid of an oxygen dissociation curve. This method of correction was also used and gave results agreeing, within the limits of the experimental error, with those obtained by interpolation. The corrections, in any case, were very slight (seldom amounting to over 5 per cent) and hence no appreciable error has been introduced in the results recorded in this paper over those obtained at sea-level.

Pulse rate determinations were made by palpation; blood pressure by auscultation and a mercury manometer: alveolar samples, by the Haldane

Priestley method, taking the samples at the end of expiration: the oxygen consumption by analysis of the expired air collected in a Douglas bag: the hemoglobin determinations by a colorimetric comparison, standardized by an oxygen capacity determination.

The cardiac output on the summit of Pike's Peak. The results obtained on A. G. and L. C. G. on the summit of Pike's Peak are given in tables 3 and 4. In the first column (tables 3 and 4) are given the day after arrival on the Peak. The subjects left Colorado Springs at the base of the mountain

TABLE 3

The results of daily measurements obtained on A. G., on Pike's Peak. altitude 14,109 feet (4,300 meters), from June 21 to July 10, 1929

DAY AFTER ARRIVAL ON PEAK	HEMOGLOBIN CONTENT	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	per cent	*per minute*	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
1		86	120/85	250	55	4.55
2		90	118/70	244	49	4.98
3	129	82	116/70	246	47	5.23
4		76	108/68	242	43	5.63
5		74	98/60	245	42	5.83
6	136	76	104/70	244	46	5.30
7		78	100/60	250	52	4.77
8		75	110/75	254	59	4.31
9	138	75	102/65	250	60	4.17
10		70	100/65	242	60	4.03
11		70	100/67	240	60	4.00
12	140	72	98/70	251	61	4.11
13		70	98/70	248	62	4.00
14		68	98/70	247	60	4.12
15	143	70	96/60	244	61	4.00
16		68	97/62	245	63	3.89
17		64	95/60	251	60	4.18
18	140	68	96/60	247	58	4.26
19		67	98/60	250	63	3.97
20	138	70	100/65	244	60	4.07

at 2:00 p.m. and arrived, by automobile, at the summit about two hours later. The evening was spent in unpacking and setting up the apparatus of the expedition. Except for breathlessness on exertion, this was accomplished with comparative ease. The data indicated in the tables as obtained on the first day after arrival on the Peak were made on the following morning, i.e., about 15 hours after arrival at the summit. Reference to table 3 shows that in the case of A. G., the cardiac output was elevated above the normal on the day following arrival at the Peak, i.e., 15 hours after the

ascent. The cardiac output continued to rise on the following days, reaching a maximum of 5.83 liters as compared to the sea-level value of 4.00 liters, or an increase of 45 per cent over the sea-level value. On the sixth day, the cardiac output had fallen and continued to decline until the ninth day, after which the sea-level values were obtained until the end of the subject's stay on the Peak.

TABLE 4

The results of daily measurements obtained on L. C. G.; on Pike's Peak, altitude, 14,109 feet (4,300 meters) from June 21 to July 10, 1929

DAY AFTER ARRIVAL ON PEAK	HEMOGLOBIN CONTENT	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	per cent	*per minute*	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
1		75	117/70	189	60	3.15*
2		86	115/68	190	56	3.39*
3	113	88	118/77	201	52	3.86
4		80	100/60	193	45	4.30
5		80	98/60	186	46	4.06
6	128	78	98/65	200	51	3.95
7		78	100/60	196	55	3.56
8		74	98/60	191	57	3.25
9	134	75	102/68	200	59	3.39
10		72	98/70	202	60	3.37
11	140	72	98/70	209	62	3.37
12		72	98/70	190	60	3.17
13		72	98/70	183	60	3.05
14		72	95/65	186	62	3.00
15	140	70	98/65	190	61	3.11
16		72	97/65	188	60	3.13
17		70	100/65	200	65	3.08
18	138	70	98/70	195	64	3.04
19		72	102/70	193	65	2.97
20	136	72	100/65	200	67	2.99

* Subject was ill in bed with torpor, anorexia and headache until noon of the second day.

Results similar to those obtained on A. G. were found in the case of the other subject studied, L. C. G. (table 4). The latter subject was confined to bed with torpor, anorexia, frontal headache, and general malaise for the first 36 hours after arrival at the Peak. She was thus subject to a mild attack of mountain-sickness without the gastric disturbances (nausea, vomiting and diarrhea) which are often accompaniments of this condition. It is interesting to note that the cardiac output during the first day, when the subject was ill, was normal, and it did not begin to rise until the second

day after arrival, or at the cessation of the symptoms of mountain sickness. Cyanosis, which is a prominent symptom on arrival at high altitudes, also persisted undiminished until the third day, whereas in the case of subject A. G. it was markedly ameliorated on the second day after arrival. This condition of affairs is consistent with the observed changes in the cardiac output. As in the case of subject A. G., the cardiac output of L. C. G. gradually rose, reaching a maximum (43 per cent above the sea-level value) on the fourth day, and then declined until the twelfth day when the normal sea-level value was again resumed.

The results on the above described two subjects, indicate a gradual rise in the cardiac output, beginning on the first or second day after arrival on Pike's Peak; a maximum increase on the fourth or fifth day; and a gradual decline to the normal value, which is again resumed on the ninth to twelfth day. As shall be shown later, these changes are related to the hemoglobin variations which are occurring at the same time and one might expect certain individual variations in the response of the cardiac output to high altitudes. In order to determine if the conclusions based on the results obtained on the two subjects, A. G. and L. C. G., were valid when applied to other individuals, determinations were made on all subjects available during the time of the stay on the mountain. As has been shown in a previous communication (Grollman, 1929e) one can predict from surface-area data, the normal cardiac output of young, healthy individuals. It is thus possible to judge, from a single determination, the degree of deviation of that determination from the normal. In order to obtain such data, however, rigid precautions must be observed. It was, therefore, necessary to pick the subjects with care and ensure the absence of any psychic or other factor which might vitiate the results. All the subjects were males in the third decade of life. The determinations were made early in the morning usually before the subject had arisen from bed. The results of measurements on 10 individuals are given in table 5. In the last column of this table are given the cardiac outputs expressed in terms of liters per square meter of body surface which were shown (Grollman, 1929e) to be equal to 2.2 ± 0.3 for the age group under consideration here.

The first 4 subjects of table 5 (B., T., J. and F.) were studied within 15 hours after their arrival on the Peak. They all showed cyanosis in some degree but presented only mild symptoms of mountain sickness. Their cardiac outputs are seen to be at the upper limit of normalcy. In the case of the fifth subject (R.) there is a definite increase of the cardiac output, on the second day after arrival, over the predicted normal value. The next three subjects (D., A. and H.) had been on the mountain for from 4 to 7 days, and their cardiac outputs show a great increase over the normal. The last two subjects (G. and M.) had been on the Peak continuously for 6 weeks except for an occasional day spent at Colorado Springs. The cardiac output of these subjects was normal.

The results of table 5 are in complete accord with the more extensive findings on subjects A. G. and L. C. G. and it seems safe, therefore, to draw the general conclusion deduced above from the results of tables 3 and 4, viz., that *the cardiac output of young normal individuals at the altitude of Pike's Peak is gradually increased during the days following arrival from a low altitude but declines again and resumes its normal sea-level value within about two weeks.*

TABLE 5

Results of measurements on normal, young individuals in the basal, resting condition, on the summit of Pike's Peak

SUBJECT	CONDITION	HEIGHT	WEIGHT	PULSE RATE	BLOOD PRESSURE	CARDIAC OUTPUT	
		cm.	*kgm.*	*per minute*	*mm. of Hg*	*liters per minute*	*liters per minute, per sq. m. of body surface*
B.	12 hours after arrival (via automobile): no marked symptoms: moderate cyanosis	176	72	78	110/65	3.95	2.10
T.	Ascended mountain by foot on preceding day: little sleep: marked cyanosis	173	61	90	122/80	4.23	2.45
J.	Ascended mountain by foot on preceding day: marked cyanosis	185	63	88	118/70	4.50	2.45
F.	First morning after arrival: sick during previous night	173	64	92	105/68	4.16	2.36
R.	Second morning after arrival: "mountain-sick" on previous day	171	59	68	118/70	4.81	2.85
D.	Fifth day after arrival: acclimatized	176	61	82	112/73	5.31	3.04
A.	Fourth day after arrival: acclimatized	168	60	80	115/70	5.23	3.11
H.	Seventh day after arrival on Peak	179	70	100	126/78	4.85	2.58
G.	Six weeks after arrival	167	54	82	108/65	3.65	2.28
M.	Six weeks after arrival	170	65	75	106/66	4.12	2.35

The relation of the cardiac output to the process of acclimatization. Previous investigators of the physiological factors involved in the process of acclimatization have accorded only a minor consideration to the rôle played by changes in cardiac output in this process. This has been due to the belief that the cardiac output was but little changed by residence at high altitudes. The results of the present investigation, however, show that this change is of sufficient magnitude to be a very important factor in

acclimatization. The fact that the cardiac output does not rise immediately on arrival at a high altitude but requires some days before it attains its maximum value, is evidence that low oxygen tension of the inspired air, as encountered at the elevation of Pike's Peak is not in itself an immediate stimulus to an increased cardiac output. As shall be shown in a later section, the degree of anoxemia encountered at Pike's Peak when produced by breathing low oxygen mixtures at sea-level does not cause an immediate rise in the cardiac output.[1] We must thus conclude that the degree of anoxemia, encountered at the altitude of Pike's Peak, whether produced by residence at high altitude or by breathing air of low oxygen content does not, *per se*, produce an immediate change in the cardiac output. The increases observed after several days are, therefore, (unless one makes the improbable assumption of a long latent period) secondary effects of other changes which take place in the organism.

We may visualize the changes occurring in the process of acclimatization, then, as consisting of a series of changes in the organism which, among other things, also results in an acceleration of the cardiac output. Although the arterial blood reaching the tissues has a lower oxygen tension than at sea-level, the mixed venous blood, due to the increased cardiac output, has approximately the same oxygen tension as at sea-level. And it is the oxygen tension of this mixed venous blood (i.e., the blood leaving the tissues) which is most probably best representative of the oxygen tension of the tissues. Hence we see that due to the increased cardiac output, the tissues themselves are maintained in a constant internal environment in so far as oxygen is concerned. However, soon after arrival at a high altitude the hematopoietic function of the organism is also stimulated and a great increase in the hemoglobin content of the blood results (column 2, tables 3 and 4). As the hemoglobin content of the blood increases the cardiac output in turn decreases, the increased oxygen capacity of the blood replacing the increased cardiac output as a means of supplying the tissues with their normal oxygen content. When the hemoglobin content reaches its maximum, the cardiac output has returned to normal. There is thus a definite relation between the *cardiac* and *hematopoietic* reactions to high altitudes, the former being more rapid in its development and being gradually replaced in its function by the latter. Both factors aim to furnish the tissues with their normal supply of oxygen and overcome any deficit occasioned by the low oxygen tension of the atmosphere.

Pulse rate changes at high altitudes. Observations on the pulse rate changes which occur at high altitudes have been made by a number of previous observers.[2] The results of tables 3 and 4 show the rise in pulse

[1] Hasselbalch and Lindhard (1915) also investigated this problem in a low pressure chamber, but their results are not definitely conclusive.

[2] Due to the voluminous nature of the literature on the effect of high altitudes on the various physiological functions such as pulse rate, blood pressure, oxygen

rate on Pike's Peak with maxima on the third day, in the case of L. C. G., on the second day, in the case of A. G., and a gradual decline thereafter. The pulse rate, however, did not resume its value at sea-level even after a month's stay on the Peak. A comparison of the values of the pulse rate and the cardiac output shows the obvious independence of these two functions. This same disparity[3] between the pulse rate and cardiac output has been found so often in physiological variations of man (Grollman, 1928; 1929b, c, e, f) that the conclusion is inevitable that *pulse rate and cardiac output often bear no simple interdependent relationship to one another nor is a change in one an indication of changes in the other.*[4] Changes in pulse rate appear to be merely symptoms of an effort on the part of the heart to accelerate the blood flow. Actual changes in the circulation are in part also dependent, however, on extra-cardiac changes (e.g., peripheral changes) and it is only after these secondary changes occur that an increased cardiac output is rendered possible, usually with an accompanying *diminution* in the pulse rate. Thus after the 2nd or 3rd day at Pike's Peak, the continued daily increase in cardiac output was accompanied by a diminution in the pulse rate, which obviously implies a marked increase in the systolic output of the heart.

Blood pressure changes at high altitudes. The blood pressure changes encountered at the elevation of Pike's Peak are not of a very great magnitude. Indeed, previous observers have considered the changes as so slight as to be within the errors of observation. The results recorded in the present paper were taken under particularly constant conditions and are, therefore, considered as significant.

As shown in tables 1 and 2 the blood pressures of the subjects studied during the present work is constant (within the errors of measurement) from day to day. This constancy has been found to apply over periods of many months, when the determinations are made, in bed, soon after the subject's awakening in the morning. The results recorded in tables 3 and 4 were obtained in this manner. The changes noted during the early days of residence on Pike's Peak are seen to be rather large although still within the limits usually ascribed to normal. The systolic pressure of both A. G. and L. C. G. was elevated about 20 millimeters above the sea-level value. This increase gradually declined until the normal sea-level value was again attained on about the fifth day after arrival on the Peak. The

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consumption, vital capacity, etc., no attempt is made in this paper to refer to all previous observers. Such references may be obtained from the reviews of Cohnheim (1903), Schneider (1921), Barcroft (1925), Loewy (1926) and Monge (1928).

[3] Gaisböck and Jarisch (1927) found a similar disparity after the administration of sodium nitrite.

[4] Changes in these functions during exercise and psychic disturbances (Grollman, 1929d) are the most obvious exceptions to this rule.

decrease in pulse pressure and in the pulse rate which occurs during the early days on Pike's Peak when the cardiac output is increasing, is explicable if we consider the very likely changes in the peripheral circulatory bed which are occurring at this time.

The alveolar tensions on Pike's Peak. The fall in the alveolar gas tensions at high altitudes has been so fully discussed by other writers (*cf.* Schneider 1921, 1923) that detailed results obtained during this investigation need not be given. The alveolar carbon dioxide tension of A. G. was 27 mm. on the day following arrival on the Peak. It gradually decreased on the following days until it reached a minimum of 23 mm. on the twelfth day after which it again showed a gradual increase during the remainder of the stay on the mountain. In the case of L. C. G. similar results were obtained except that the initial tension (28 mm. on the day following arrival on the Peak) showed a continual decrease to 23 mm. on the nineteenth day which was sustained thereafter.

TABLE 6

Vital capacity determinations

SUBJECT	LOCALITY	VITAL CAPACITY
		cc.
A. G.	Baltimore	3,930
	Pike's Peak	3,490
	Manitou	3,960
L. C. G.	Pike's Peak	2,400
	Manitou	2,280

Vital capacity changes. The effect of residence at high altitude on the vital capacity has been studied by a number of observers. Zunz and his co-workers (1906), Durig (1909) and Monge (1928)f ound a decrease in the vital capacity at high altitudes which has been variously attributed to the expansion of intestinal gases, to fatigue of the respiratory muscles, or to an increased muscle tone due to low temperatures. Barcroft *et alii* (1923) found no remarkable alteration in the vital capacity at Cerro de Pasco, with a decrease in 4 individuals and a slight increase in one subject. The results obtained on the 2 subjects of this study are shown in table 6.

Basal metabolism at high altitudes. The basal metabolism of individuals resident at an altitude above 14,000 feet has been variously found by different observers to either be unchanged or somewhat increased. The results of the present investigation (tables 3 and 4) show a small but definite increase in the oxygen consumption, in the basal condition as compared to the sea-level values.

The possible relationship of this metabolic change to changes in the

activity of the thyroid may be inferred from the recent investigations of Mark (1929).

The effect of the ingestion of food, exercise, and temperature on the cardiac output at high altitudes. Normally, healthy acclimatized persons when at rest on Pike's Peak, do not experience any subjective symptoms indicative of the low oxygen tension to which they are subjected. However, during exercise, or after the ingestion of a heavy meal there result certain subjective symptoms—chiefly breathlessness—which are characteristic of life at high altitudes. It was, therefore, thought of interest to investigate the cardiac output during these conditions in order to determine if this function was altered in a different manner at high altitudes than at sea-level. The results during mild exercise will be reported in a future paper dealing with cardiovascular reactions to muscular exercise. The effect of a heavy meal

TABLE 7

A comparison of the effect of the ingestion of a heavy meal on the cardiac output at low and high altitudes

SUBJECT	DATE	LOCALITY	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	1929		*per minute*	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
A. G.	VI.30	Pike's Peak	93	102/70	318	62	5.13
A. G.	VII.1	Pike's Peak	92	102/72	296	58	5.10
A. G.	VII.12	Pike's Peak	96	102/78	356	65	5.48
A. G.	VII.23	Manitou	86	95/65	365	65	5.61
A. G.	VII.24	Manitou	86	95/58	377	66	5.71
L. C. G.	VII.2	Pike's Peak	92	98/62	290	60	4.83
L. C. G.	VII.26	Manitou	84	90/60	294	58	5.07

on the cardiac output on Pike's Peak as compared to similar determinations made at Manitou are recorded in table 7. All the determinations were made one hour after the ingestion of food, the subject having rested in the meantime comfortably relaxed. The subjects, at the time of the experiments, were completely acclimatized. It has been previously shown (Grollman, 1929c) that after the ingestion of food the cardiac output rises rapidly, reaches a maximum (within an hour), and remains elevated for some time. The arterio-venous oxygen difference either remains unchanged (in which case the cardiac output is proportional to the metabolism) or drops somewhat. The results of table 7 show that the cardiovascular reaction to food is essentially the same at high altitudes as at sea-level. The difference in subjective response and the breathlessness are thus occasioned solely by the extra strain on the organism due to the increased metabolism and its accompaniments resulting from the ingestion of food.

Unacclimatized persons often show marked reactions (nausea or fainting) after a heavy meal or after being subjected to a high temperature. This phenomenon is easily explained when we consider the increase in cardiac output caused by the ingestion of food or by increasing the external temperature. At high altitudes the limits of variation in this function which will produce symptoms are narrowed due to the already extreme conditions brought about by the low oxygen tension of the inspired air. Hence, any factor, such as a heavy meal or increased external temperature, tends to produce the same symptoms as does mild exercise. After the ingestion of a heavy meal, the effect of exercise is particularly noticeable. This is to be attributed to the fact that the effect of food and exercise on the cardiac output are summated as was shown by Jarisch and Liljestrand (1927). This summation was also found to occur on Pike's Peak in a few experiments conducted during the present expedition.

The relation of mountain sickness to the cardiac output. Investigators of the physiology of high altitudes have elaborated numerous theories as to the cause of mountain-sickness (for a review of which, consult Monge, 1928). Due to the lack of reliable data on the cardiac output at high altitude this function has not entered into consideration of the problem. The results of the present study indicate, however, the possible existence of of a relationship between the cardio-vascular response to diminished oxygen tension and the manifestations of mountain sickness. Data available at present are insufficient to permit a conclusion regarding the relation of anoxemia of the tissues to the state of well-being of the subject. However, the relation of the arterio-venous oxygen difference of the blood has been found in the case of the malaise following typhoid vaccine injection (Grollman, 1929, f) and in unpublished experiments on the cardiac output during general malaise, to be reflected in the subjective feelings of the subject—malaise being accompanied by a high arterio-venous oxygen difference. The suggestion, therefore, presents itself that the mild form of mountain sickness (such as occurred in the subjects whom we had the opportunity of observing on Pike's Peak) is merely a manifestation of the diminished oxygen tension of the mixed venous blood (which is equivalent in its effect on the tissues to a higher arterio-venous difference at sea-level). As the cardiac output increases, this "oxygen-hunger" disappears and with it also the symptoms of torpor, malaise, anorexia, etc., which constitute mountain sickness.

The possibility of an hypertrophy of the heart resulting from a long stay at a high altitude is not supported by the data of the present paper. Except for the initial period of acclimatization during which the cardiac output is increased, the heart is not subjected to any constant increased effort which would result in a compensatory hypertrophy.

The cardiac output after the return to lower altitudes. Because of the

marked physiological changes produced by residence at high altitudes, it appeared of interest to investigate the circulatory changes following return to a lower altitude. Consequently, after a month's residence on Pike's Peak, the experimental determinations described above were resumed at Manitou, Colorado, which is situated at the base of Pike's Peak. Unfortunately Manitou is 6562 feet above sea-level. Due to the gradual rise of the plains which give rise to the Rocky Mountain formation, one has to travel a long distance from Pike's Peak to reach sea-level areas. The rapidity with which circulatory adjustments follow changes in altitude

TABLE 8

The results of daily measurements obtained on A. G. at Manitou, Colorado, altitude approximately 6,562 feet (2,000 meters), after the descent from Pike's Peak

DAY AFTER DESCENT FROM PEAK	HEMOGLOBIN CONTENT	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	per cent	*per minute*	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
1		64	98/65	240	60	4.00
2		68	104/70	242	66	3.67
3		60	98/68	250	67	3.73
4	112	56	96/65	244	61	4.00
5		64	100/75	246	57	4.32
6		60	98/70	240	58	4.15
7	115	60	98/68	243	60	4.05
8		62	97/65	244	61	4.00
9		60	95/60	240	60	4.00
10	118	63	97/62	248	60	4.13
11		64	100/65	240	62	3.84
12		58	98/65	245	63	3.89
13	105	62	97/65	246	65	3.78
14		63	100/67	242	60	4.03
15		65	98/63	240	58	4.14
16	112	60	98/65	244	57	4.28

necessitated, however, making determinations soon after descending the Peak, and hence it was impossible to continue the work at actual sea-level.

The determinations made at Manitou are recorded in tables 8 and 9. The subjects of the experiments left Pike's Peak by train at noon arriving at Manitou several hours later. The experiments were then resumed on the following morning in the usual manner.

The cardiac output of A. G. (table 8) shows a slight decrease on the first two days at Manitou: that of L. C. G. (table 9) shows no change from the values on the Peak or the normal values obtained at sea-level. The reaction of these two subjects to the descent differed. A. G. suffered no subjective symptoms; L. C. G., on the other hand, was extremely drowsy and

torpid for the first 30 hours after the descent. Drowsiness is a very common symptom of which persons resident on the Peak for some time often complain on their descent to lower altitudes. The observed changes on A. G. are too small and of too short duration to permit definite conclusions. Nevertheless, the different reactions of this subject and L. C. G. to the change in altitude, suggest a possible diminution in cardiac output, analogous in its behavior to the increase noted on the ascent. Changes at sea-level would probably have been more striking.

TABLE 9

The results of daily measurements obtained on L. C. G. at Manitou, Colorado, altitude, approximately 6,562 feet (2,000 meters), after the descent from Pike's Peak

DAY AFTER DESCENT FROM PEAK	HEMOGLOBIN CONTENT	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
	per cent	*per minute*	*mm. of Hg*	*cc. per minute*	*cc. per liter*	*liters per minute*
1		64	98/65	190	63	3.02*
2		66	94/64	190	60	3.17
3		63	93/65	200	65	3.08
4	120	52	92/60	218	68	3.21
5		65	93/65	214	65	3.29
6		61	95/68	175	56	3.13
7	115	55	90/60	177	59	3.00
8		60	95/66	180	60	3.00
9		61	95/65	201	62	3.24
10	114	63	93/63	203	63	3.22
11		58	92/58	180	60	3.00
12		56	92/62	205	63	3.25
13	115	60	92/60	199	66	3.02
14		60	92/60	194	61	3.18
15		58	93/57	198	61	3.25
16		62	93/57	199	61	3.26

* Subject extremely drowsy for 24 hours after the descent.

The extremely slight changes observed at Manitou were surprising in view of the marked increases after ascent to Pike's Peak. It might be expected that the reversal of the conditions encountered at the two altitudes might lead to a marked diminution in cardiac output. However, reference to the hemoglobin values shows that the concentration of this substance (which would be the chief cause of a decreased cardiac output) drops in an extremely short time. The mechanism of this decrease in hemoglobin concentration is unproven. It seems improbable that so great a quantity of hemoglobin could be destroyed in the body in so short a time without marked manifestations of this destruction (such as jaundice), and hence it would appear that the hemoglobin is removed from the circulation and

stored within the spleen, bone marrow, etc., from which it gradually disappears.

The pulse changes observed at Manitou do not show a decline to subnormal values which has been claimed to occur after long residence at high altitudes (Schneider, 1921).

In order to visualize the cardio-vascular effects of residence at high altitudes, the results of the present study, as found on subject A. G., have been graphically represented in figure 1. The graph shows the cardiac output at sea-level, Pike's Peak, and Manitou and the accompanying

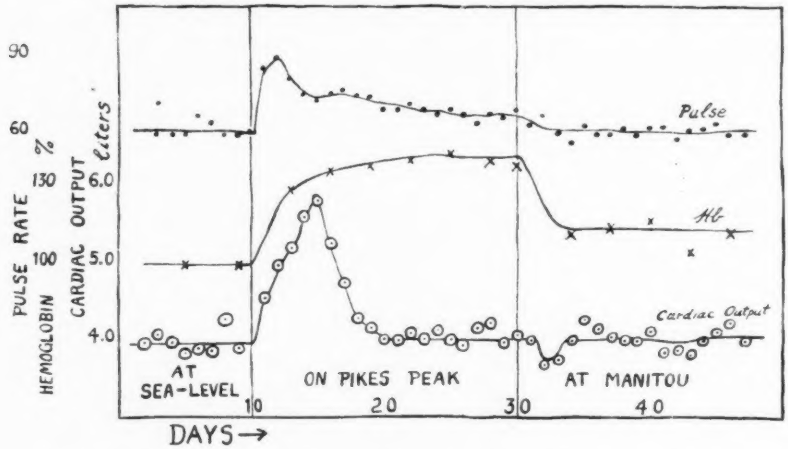

Fig. 1. The cardiac output, hemoglobin content of the blood, and pulse rate of A. G. at sea-level, and the changes in these functions observed on ascending to the summit of Pike's Peak and subsequently descending to the base of the mountain. The abscissae represent days; the ordinates, the various observed functions. The graph represents the subject as ascending Pike's Peak on the 10th day and descending to Manitou on the 30th day. As a matter of fact, the sea-level determinations were made some weeks before ascending the Peak and the determinations at Manitou were made after a month's stay on the Peak.

changes in the pulse rate and hemoglobin content of the blood, at these places.

The effect of anoxic anoxemia on the circulatory system. As previously stated, the results obtained on the summit of Pike's Peak are to be attributed most probably to the low oxygen tension of the inspired air. It appeared interesting, therefore, to determine the effect on the circulation of anoxic anoxemia, as produced by breathing mixtures of air and nitrogen, at sea-level. The results of this study are shown in the accompanying table (table 10) and fig. 2. The experiments were conducted at Baltimore

TABLE 10

The effect of anoxic anoxemia, produced by breathing mixtures of air and nitrogen at atmospheric pressure, on the cardiac output of A. G.

DATE OF EXPERIMENT	OXYGEN CONTENT OF INSPIRED GAS (DRY)	OXYGEN TENSION OF INSPIRED GAS	OXYGEN TENSION OF ALVEOLAR AIR	DURATION OF REBREATHING PERIOD	PULSE RATE	BLOOD PRESSURE	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT	INCREASE IN CARDIAC OUTPUT OVER NORMAL
1929	*per cent*	*mm. of Hg*	*mm. of Hg*	*minutes*	*per minute*	*mm. of Hg*	*cc. per liter*	*liters per minute*	*per cent*
September 10	20.93	151	115	—	76	105/65	46	6.09	—
	17.66	128	70	8	76	102/65	46	6.09	0
	15.14	109	62	8	80	102/69	46	6.09	0
	11.34	82	47	8	88	108/64	40	7.00	15
September 11	20.93	157	112	—	60	107/66	56	4.82	—
	13.90	105	61	10	76	111/65	56	4.82	0
	11.09	84	42	13	80	111/65	46	5.87	22
	7.02	53	26	9	120	118/70	26	10.42	116
	20.93	157	112	15	58	117/81	50	5.40	12
September 14	20.93	153	114	—	72	109/64	52	5.00	—
	12.47	92	55	30	84	115/68	52	5.00	0
	10.53	78	44	30	84	113/68	41	6.34	27
	9.56	71	33	30	92	114/69	30	8.67	73
October 1	20.93	155	106	—	60	108/76	65	3.87	—
	99.4	757	648	15	60	108/75	66	3.82	0
	99.4	757	660	30	54	113/77	65	3.94	0
	99.4	757	661	45	55	111/81	68	3.71	0
	20.93	155	107	15	62	115/82	64	3.94	0
	20.93	155	109	30	61	113/85	65	3.87	0
October 24	20.93	158	110	—	62	109/74	60	3.93	—
	11.53	87	46	10	76	113/71	55	4.29	7
	11.53	87	47	25	76	110/69	53	4.45	11
	11.53	87	44	45	76	108/72	54	4.37	9
	20.93	158	110	15	62	108/74	58	4.07	—
November 2	20.93	159	112	—	60	112/83	57	4.00	—
	9.93	75	40	10	80	115/84	46	5.17	29
	9.93	75	36	25	80	118/82	42	5.67	42
	9.93	75	35	50	80	119/85	43	5.53	38
	20.93	159	114	15	58	114/83	59	4.03	—
November 7	20.93	160	102	—	62	108/76	62	3.81	—
	8.85	68	35	7	80	112/75	39	6.05	55
	8.85	68	32	15	84	112/75	37	6.37	63
	8.85	68	31	30	84	110/70	38	6.21	57
	8.85	68	29	45	86	108/73	35	6.83	75
	8.85	68	32	60	88	105/68	38	6.21	57
	20.93	160	105	20	60	108/74	60	3.93	—

(sea-level) two months after the descent from Pike's Peak. The mixtures to be rebreathed were prepared by compressing nitrogen and atmospheric air in a tank and leading the resulting mixture into a Douglas bag from which it was inspired. The oxygen content and tension of the dry inspired gas is shown in the second and third columns of the table. By means of a series of Lovén valves, ordinary three-way taps, Sadd-valves, etc., it was possible to carry out all the necessary procedures without ceasing to breathe the experimental mixture.

The following conclusions may be drawn from the results of the experiments cited in table 10. The pulse rate is increased when the oxygen tension of the inspired gas is lowered. Breathing pure oxygen (experiment of Oct. 1) decreases the pulse as compared to breathing atmospheric air, and a reduction of the oxygen tension of the inspired air to about 110 mm. results in an increase in the pulse rate (Sept. 10) which may be doubled (Sept. 11) at extremely low oxygen tensions. A change in pulse rate is not necessarily accompanied either by a change in the blood pressure (column VII) or of the cardiac output (column IX). Changes in the cardiac output do not occur until the oxygen content of the inspired air is about 11.6 per cent, below which point the cardiac output rises rapidly with further decrease in the oxygen content of the inspired air. There is thus a threshold point, below which anoxic anoxemia results in an immediate rise in the cardiac output. This point corresponds to the conditions encountered at an altitude of about 15,000 feet (4572 meters). One might, therefore, conclude that on ascending to this altitude rapidly (e.g., in aeroplane flights) there is an immediate increase in the circulatory rate. The delayed rise in the cardic output which was found at the altitude of Pike's Peak (14,109 feet) would thus occur sooner at more elevated regions. Moreover, it is quite probable that a similar delay in the change of the circulation would be found at sea-level if the breathing experiments described in table 10 could be continued over a period of days or in closed-chamber experiments such as were conducted by Hasselbalch and Lindhard (1915).

The arterial saturation at the point where an increased cardiac output results is about 83 per cent, at which point, if no acceleration of the cardiac output would occur, the coefficient of oxygen utilization would be 36 per cent (instead of the normal 32 per cent) and the oxygen tension of the mixed venous blood would be about 27 mm. (instead of the normal 36 mm.).

The fourth experiment of September 11 is instructive because of the low oxygen tension of the inspired gas, which corresponds to an altitude of about 8000 meters (29,000 feet). The cardiac output was increased 116 per cent after 9 minutes' breathing of this mixture. The subject showed marked symptoms of anoxemia during the experiment, extreme cyanosis and loss of mental capacities. The alveolar oxygen tension during this

experiment was only 26 mm. which corresponds to about 35 per cent saturation of the arterial blood. The oxygen utilization was 41 per cent which is only about 8 per cent above the normal. The return of the circulatory rate to normal after the cessation of this experiment was slow, for as shown in the next experiment (last experiment of Sept. 11), the cardiac output and blood pressure were still increased 15 minutes after the subject resumed breathing atmospheric air. The pulse, on the other hand, had returned to normal at this time.

The first three experiments of table 10 were not done in the basal condition, as may be noted from the values of the cardiac output when the

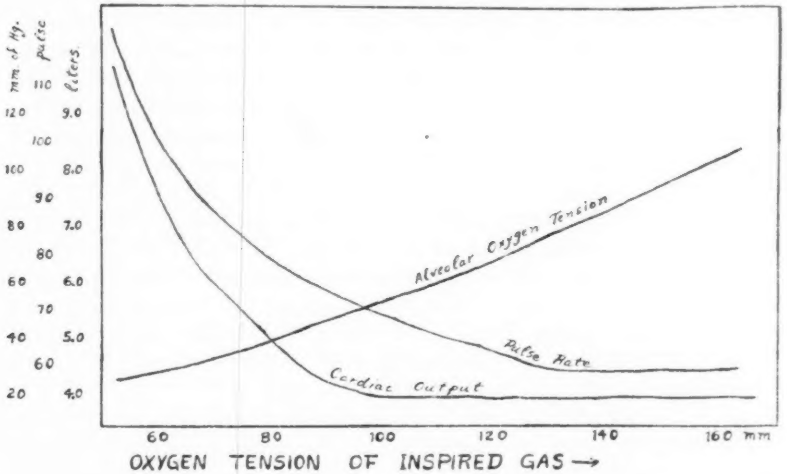

Fig. 2. The relation of the oxygen tension of the inspired air to the cardiac output (liters per minute), pulse rate (per minute), and alveolar oxygen tension (millimeters of mercury) as observed on subject A. G. at sea-level.

subject was inspiring atmospheric air. The remainder of the experiments were conducted in the basal condition. Comparison of the two sets of data indicates that the effect of diminished oxygen tension on the cardiac output is summated on an increase already present due to other causes. This is similar to the additive effect of exercise on the cardiac output after the ingestion of food, which has been discussed above.

The last three experiments quoted in table 10 (Oct. 24, Nov. 2, and Nov. 7) show that the increased cardiac output resulting from inspiring mixtures of low oxygen content, develops rapidly. There is no demonstrable increase in the cardiac output after breathing a given mixture for an hour, over the cardiac output obtained 7 minutes after beginning the

rebreathing. It would thus appear that *there are two essentially different conditions which bring about a cardiac response to anoxemia.* There is an *immediate* reaction which shows itself in the course of a few minutes, and a *delayed* reaction (such as was observed on Pike's Peak) which requires many hours for its development. The first of these reactions is probably a direct effect of anoxemia on the higher centers. The delayed reaction, on the other hand, is probably dependent for its development on slowly occurring changes in the tissues.

The experiments of October 1 indicate that breathing almost pure oxygen, as compared to air, does not appreciably affect the cardiac output. This is not surprising when one considers the fact that due to the parabolic shape of the oxygen dissociation curve of blood, any increase in the alveolar oxygen tension above about 100 mm. will affect the oxygen content of the blood only in so far as it increases the amount of oxygen in physical solution in the blood. Hence, despite the six-fold increase in the oxygen content of the inspired air, the blood reaching the tissues is only about 10 per cent richer in oxygen, and it is this latter tension which would be expected to cause any change in the cardiac output. Unfortunately the determination of the cardiac output when breathing pure oxygen is fraught with greater errors than when air is breathed. This is due to the greater analytical errors involved and the necessity of making corrections for the deviation of the alveolar oxygen tension during the rebreathing period from the oxygen tension before the determination (as has been discussed above). Hence one cannot detect slight changes produced by the breathing of pure oxygen with the present experimental methods.

In connection with the above results, reference may be made to the experiments on the effect of anoxemia on the cardiac output of the lower animals. Doi (1922) found that the cardiac output of urethanized cats was unchanged when the oxygen content of the inspired air was as low as 13.3 per cent. This is in accord with the present findings. Harrison and Blalock (1927) found an increase in the cardiac output of normal dogs when the arterial blood was less than 70 per cent saturated with oxygen. This threshold value is somewhat lower than the results of the present investigation, but examination of the data presented by Harrison and Blalock shows such extreme variations and inconsistencies that their conclusion is open to rather severe criticism.

The experiments of Doi, quoted above, have been used as evidence for the view that the cardiac output is unchanged at high altitudes. However, as shown above, experiments of short duration, conducted at sea-level tell us nothing of the changes in cardiac output which occur after some hours or days at high altitudes. The results of the present investigation also show the importance of the duration of the subject's stay at a high altitude in determining the value of the cardiac output. This time factor has been entirely ignored by previous investigators.

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I am deeply indebted to L. C. G. who not only endured the long sojourn on the mountain to act as a subject for the experiments, but also ably assisted in their performance.

SUMMARY

The effect of residence at high altitudes on the cardiac output and its related functions was investigated by comparing the results of measurements at sea-level with those obtained on the summit of Pike's Peak. The cardiac output gradually increased on the summit of Pike's Peak, reaching a maximum of about 40 per cent above its sea-level value on the fifth day after arrival on the Peak. It then gradually declined again to its normal value. The relation of this change to the hemoglobin content of the blood was demonstrated. The changes of the pulse rate, blood pressure, basal metabolism, alveolar gas tensions, and the effect of the ingestion of food on the cardiac output at high altitudes was also studied and the relation of these various factors to other physiological changes, discussed. After a month's stay on Pike's Peak, a study of the cardiovascular changes encountered on returning to a lower level was made.

The relation of the oxygen content of the inspired air to the cardiac output was determined after breathing gas mixtures of varying oxygen content, for varying periods of time at sea-level. The existence of a critical concentration of oxygen below which further reduction in the oxygen content of the inspired air causes an immediate increase in the cardiac output was demonstrated. The relation of this immediate increase to the progressively developing increase observed at high altitudes was discussed.

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THE RELATION OF THE SYMPATHETIC NERVOUS SYSTEM TO THE CONTRACTIONS AND FATIGUE OF SKELETAL MUSCLE IN MAMMALS

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All attempts to show that stimulation of the sympathetic nerves produces any change of state in the resting skeletal muscle have been without result. Recent experiments of Orbeli (1923) and his student Ginetzinsky (1922), however, have shown that stimulation of the sympathetic nerves diminishes or delays the onset of fatigue in contracting skeletal muscles. Using the bloodless hind legs of frogs, they stimulated the 8th and 9th anterior lumbar spinal roots with a series of single shocks and recorded the isotonic contractions of the gastrocnemius. At intervals the abdominal sympathetic chain was stimulated at the level of the 7th ganglion. While the muscle was fully active and responding well, the superadded stimulation of the sympathetic fibers was without definite effect, but, when fatigue began to develop, sympathetic stimulation caused a definite increase in the muscular response, beginning gradually after a latent period and outlasting the sympathetic stimulation. The same beneficial effect of sympathetic stimulation was obtained if the muscle was contracting isometrically with short periods of tetanus. Later experiments of Ginetzinsky, reported by Gantt (1927), showed that similar results were obtained in an atmosphere of hydrogen, leading to the conclusion that this effect of the sympathetic impulses could not be explained exclusively "by sympathetic augmentation of oxidation of the products of metabolism." Further experiments, undertaken to discover the nature of this sympathetic effect, led Orbeli (Gantt, 1927) to the following summary of his work. "The sympathetic nervous system exerts a profound influence over the physiochemical changes occurring in skeletal muscle, accompanied by a modification of the functional ability of that muscle. These changes influence, it seems, the conditions of the motor endplate, calling forth transformations in the efficiency of the corresponding muscles. This forms a sort of regulatory mechanism for the expenditure of muscle strength and governs the condition of impulses by the motor nerves."

Wastl (1925) repeated Orbeli's original experiments on cats with intact circulation, recording the isotonic contractions of the muscle tibialis

anticus stimulated through the 6th and 7th lumbar anterior spinal roots. Stimulation of the abdominal sympathetic chain below the 5th ganglion produced either no effect or a decrease in the height of the contractions, this latter being attributed by Wastl to vasoconstriction. Wastl then repeated Orbeli's isotonic experiments on frogs, also with negative results.

Of slightly different character are the experiments reported by Nakanischi (1927). He cut the 8th and 9th spinal nerves in frogs close to the cord and the sympathetic chain below the 6th ganglion and recorded the isotonic contraction of the bloodless gastrocnemius produced by short periods of tetanic stimulation of these spinal nerves. If the spinal nerves were stimulated peripheral to the rami (i.e., the sympathetic as well as motor fibers being stimulated), the height of the contraction was greater than when the spinal nerves were stimulated central to the rami (i.e., just the motor fibers). The same increased contraction height was also obtained if the sympathetic nerves were stimulated directly and simultaneously with the spinal nerves central to the rami.

In addition to these stimulation experiments, the relation of the sympathetic nerves to fatigue in skeletal muscle has been studied in a few instances by extirpating the sympathetic nerves on the theory that, if there are sympathetic fibers to skeletal muscle controlling fatigue, destruction of the sympathetic innervation should result in a more rapid onset of fatigue in the sympathectomized muscle.

Hunter (1925) in his work on tone claimed that, after removal of the sympathetic nerves to one wing in birds, a flight of 100 meters caused marked abduction and drooping of the sympathectomized wing as compared with the normal. After removal of the sympathetic nerve supply to both wings, several short flights exhausted the birds and caused both wings to droop, thus indicating a more rapid onset of fatigue after sympathetomy.

Kuntz and Kerper (1924) removed the lumbar sympathetic trunk on one side in dogs and compared the contractions of the gastrocnemii when stimulated with a tetanizing current through the sciatic nerves. The initial contractions were equal but the gastrocnemius on the sympathetomized side fatigued much more quickly than the normal. This result was obtained immediately following the removal of the sympathetic trunk or after a period of 10 to 36 days. Ligation of the common iliac arteries did not alter the result.

Negative results, following sympathectomy, however, have been reported by several experimenters.

Tower (1926), in connection with other experiments, removed the stellate ganglion unilaterally in each of seven dogs. After a period of 5 to 7 months these dogs were trained to run in a treadmill. From a comparison of the normal and sympathectomized limbs of these animals, she concluded that "the capacity for prolonged muscular work and the onset and severity of the fatigue were in no way affected by sympathectomy."

Campos, Cannon, etc. (1929), in studying the conditions affecting the capacity of dogs for prolonged muscular work, reported that bilateral removal of the abdominal sympathetic chain in one dog and bilateral removal of the stellate ganglia in another dog in no way affected their capacity for muscular work when run in a treadmill.

Hoffman and Wertheimer (1927), in a study of strychnine convulsions in frogs, reported that sympathectomized muscles fatigued less rapidly and that their contractions were stronger and better sustained than the normally innervated control muscles.

The author's interest in this problem had its origin in an attempt to find an explanation for the fact that at high external temperatures the power of the muscles to do work is greatly reduced. If Orbeli's results are correct this effect might possibly be due to a reflex inhibition of the action of the sympathetic fibers on the muscles. In view of the conflicting evidence reported above and the need of more extensive experiments on mammals, it was decided to make an independent study, in cats, of the effect of sympathetic stimulation on the contractions and fatigue of skeletal muscle.

Methods. The experimental method was similar to that described by Orbeli and Wastl. The anterior spinal roots of the 6th and 7th lumbar nerves were stimulated in cats, under urethane anesthesia, and the isotonic contractions thus produced in the tibialis anticus muscle recorded. While the muscle was contracting, the abdominal sympathetic chain was stimulated and the resulting effect on the contractions noted.

Glass shielded electrodes, as described by Sherrington (1909), were used for stimulating the somatic and sympathetic nerves to avoid spread of current. The 6th and 7th anterior roots were stimulated together, chiefly by single break shocks at a constant rate, usually between 120 and 180 per minute depending on the condition of the animal. This was accomplished by connecting the inductorium with a motor-driven apparatus similar to that designed by Campbell (1888). The resulting curve of the muscle contractions showed the usual form, consisting of a decline in height at first, a fairly steady level, which was maintained sometimes for hours, and the final gradual decline to complete fatigue. The sympathetic chain was stimulated at intervals during the various phases of the contraction curve.

In some few experiments, in place of the series of single contractions, the effect of sympathetic stimulation was studied on short periods of tetani, lasting from 15 seconds to 1½ minutes, and alternating with rest intervals of 1 to 15 minutes, the duration of the contractions and rest periods depending on the experiment. Normally in a series of tetani there is a decrease in the initial height and a more rapid onset of fatigue in each succeeding period of tetanus. To take this factor into account, three periods of tetani of equal duration were each followed by a given interval of

rest. The sympathetic stimulation was begun either simultaneously with, or during, the second period of tetanus, or during the preceding interval of rest. By comparing this second tetanus record with the records preceding and following, the effect of the sympathetic could be clearly seen. Sympathetic stimulation was considered to have a positive result, not only when there was an actual increase in the second tetanus curve, but also when the normal decrease did not appear.

The sympathetic chain was cut and stimulated just below the 5th ganglion. At this point all the sympathetic fibers to the lower leg would be reached, as there are usually no white rami communicantes passing to the sympathetic ganglia from the lower lumbar spinal nerves. In general, the sympathetic fibers were stimulated with a tetanizing current, usually for 15 or 30 seconds, although occasionally for longer periods, and of such a strength as to cause a prompt rise of the tail hairs, and yet never strong enough to produce any visible spread of current. In some few experiments the sympathetic fibers were stimulated with single break shocks of various strength and at different speeds, in place of the tetanizing current.

The muscle was attached to a light isotonic muscle lever by a heavy thread tied to the tendon, and was free weighted with 35 to 130 grams, depending on the condition of the muscle and the type of experiment. An iron rod, inserted through the lower end of the femur while the cat was in deep anesthesia, held the limb in an immovable position. The muscle lever was arranged to record on a smoked drum, together with a time marker and a signal magnet registering the stimulation of the sympathetic chain.

In some experiments, also, plethysmograph tracings were made of the volume of the contracting muscles. The skin was removed from the right leg and a tube 8 inches long and $1\frac{3}{4}$ inches in diameter, fitted at one end with a piece of rubber tubing and at the other with a stopper, was drawn up over the foot and leg as far as the knee joint. The rubber tube fitted loosely in order not to compress the blood vessels and was made air-tight with vaseline. The tube to the tambour opened into the plethysmograph through the rubber stopper. During the last few experiments it was desirable to obtain a record of the muscle contractions simultaneously with the plethysmograph tracing. To arrange this, the thread from the tendon to the lever was run through a small glass funnel, which was inserted into the stopper. The funnel was packed with vaseline in order to avoid air leakage.

When blood pressure tracings were desired, they were taken from the carotid or femoral artery by a mercury manometer.

The effect of sympathetic stimulation on the muscle contractions was studied, not only when the normal blood supply was intact, but also

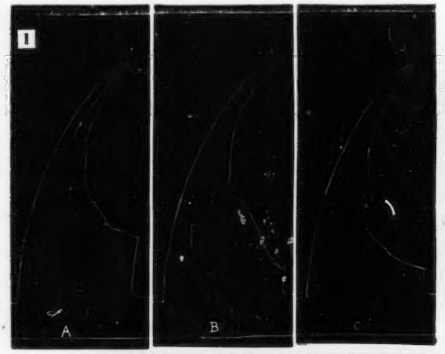
with an isolated circulation supplied to the muscles of the lower leg by cross circulation from a donor cat. The blood was supplied from the carotid artery of the donor cat to the femoral artery in the experimental cat and returned by way of the femoral vein to the external jugular. Heparin, 20 mgm. per kilo of body weight, was injected intravenously into each cat. This was repeated two or three times at hourly intervals in the donor cat. To prevent collateral circulation to the leg, all of the thigh muscles were either dissected or cut and the femur was sawed in two, while the animal was in deep anesthesia. Bleeding was prevented by tying off all exposed blood vessels and cut surfaces of muscles, and packing cotton into the shaft of the femur. By this method there was no connection whatever between the lower leg and the body of the experimental cat except the nerves. In these experiments the skin was always removed from the leg and foot so that any effect of the sympathetic was attributable to an effect on the muscle or its blood vessels.

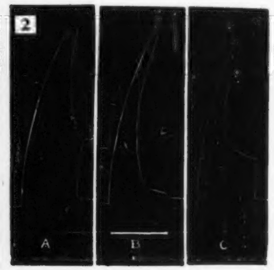
Data and results. There were 46 experiments, 37 with the circulation intact and 9 with the blood supply to the contracting muscles supplied by cross circulation from a donor cat. Plethysmograph tracings were made in 11 of the experiments and blood pressure records in three. The data and results from the experiments with intact circulation will be considered first.

A. Experiments with normal circulation. In 7 experiments the effect of sympathetic stimulation was studied on the height and shape of the curve of short periods of tetanus. Five of these 7 experiments showed better sustained contractions following sympathetic stimulation, as shown in graph 1B, where the effect of sympathetic stimulation on the tetanus can be seen by comparison with the preceding and following normal records (A and C). The improvement always began after a definite latent period. In the other two experiments there was a distinct fall in the contraction level following sympathetic stimulation (graph 2). The results were repeated several times in each experiment. When the sympathetic stimulation began simultaneously with the contraction there was no effect on the initial height due to the latent period. In three experiments, however, the sympathetic fibers were stimulated during the rest interval preceding the tetanus, with the result that two showed an increase (graph 3) and one a decrease in the initial height, when compared with the preceding and following normals. From these tetanic experiments it was evident that there were two opposing effects which might result from sympathetic stimulation, an improvement in the muscle contractions or a depression of the contractions.

Although the tetanic experiments gave interesting results, a curve of simple contractions was found much more suitable for studying the sympathetic effect. While the muscle was contracting regularly due to re-

peated single induction currents applied to the 6th and 7th anterior spinal roots, the sympathetic chain was stimulated with a tetanizing current and the resulting effect on the height of the contractions studied. Of the 24 experiments of this type, 8 showed an increase in the contraction height with sympathetic stimulation (graph 4). This effect began after a latent period and gradually increased to a maximum, which was reached after sympathe-





Graphs 1 and 2. The effect of stimulating the abdominal sympathetic chain on the tetanic contractions of the m. tibialis anticus, stimulated through the 6th and 7th anterior spinal roots. A and C in each case are normal control records. B in each case is tetanus with sympathetic stimulation. Graph 1 shows better sustained tetanus following sympathetic stimulation. Graph 2 shows a decrease in the tetanus following sympathetic stimulation.

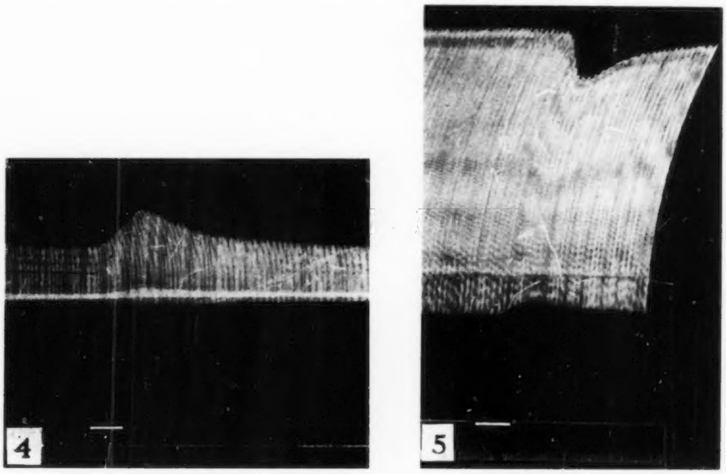
Graph 3. The effect of sympathetic stimulation on the initial height of the tetanus. Sympathetic chain stimulated between tetanus A and B. A and C are normal control records.

tic stimulation had ceased, if that was short lasting. When the sympathetic stimulation was continued for longer periods, for example one minute, the contractions remained at the higher level until cessation of the stimulation. In most cases the contractions returned gradually to their original height but in some instances they remained at the higher level. This result of sympathetic stimulation was obtained during all phases of the

contraction curve, immediately after the beginning of the contractions, during the initial decline in the contraction height, during the fatigue level, and when the muscle was almost completely fatigued.

Nine experiments showed a gradual decline in the height of the contractions following sympathetic stimulation, as can be seen in graph 5. This also began after a latent period and continued for a short time after cessation of sympathetic stimulation. The contractions then gradually returned toward normal, although in many cases they remained lower.

Seven experiments showed both results, either at different times during the experiment, or a short lasting increase followed immediately by a



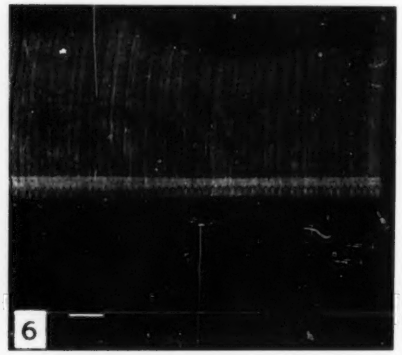
Graphs 4 and 5. The effect of stimulating the abdominal sympathetic chain on the contraction curve of the m. tibialis anticus, stimulated by single induction shocks to the 6th and 7th anterior spinal roots. Graph 4 shows an increase and graph 5 a decrease in the contraction height.

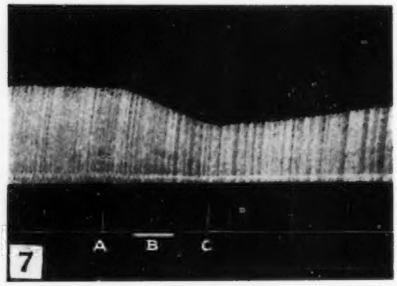
decrease (graph 6). It seemed possible that these two opposite results might be differentiated by varying the strength and speed of sympathetic stimulation. Increasing the strength of the tetanizing current above the threshold up to a certain point resulted merely in a greater response, either an increase or decrease in the contractions as the case might be. Stimulation of the sympathetic chain with single break shocks of various strengths and speeds from 20 to 310 per minute either produced no effect at all or, if sufficiently rapid and strong, a very slight effect on the contraction height. The result of sympathetic stimulation, therefore, did not vary with the strength or speed of stimulation. From these experiments

it was evident that in some cases sympathetic stimulation caused a strengthening of the muscle contractions or a temporary diminution in fatigue, while in others it produced an opposite effect.

These results of sympathetic stimulation could not be attributed to a spread of current for the following reasons: the effect appeared gradually after a latent period; it outlasted the sympathetic stimulation; it appeared in contractions which began after cessation of the sympathetic stimulation, such as was found when the sympathetic fibers were stimulated between two periods of tetanus; and it could not be imitated by replacing the sympathetic chain with a moist thread in contact with the electrodes.

Since the circulation was intact it was necessary to determine whether





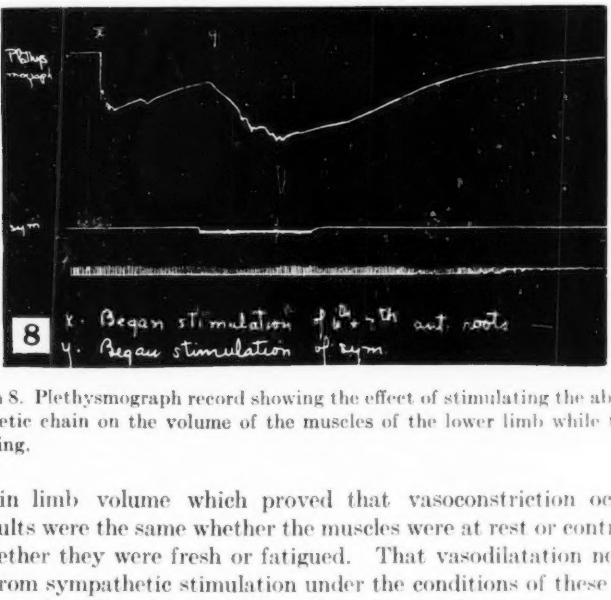
Graph 6. The effect of stimulating the abdominal sympathetic chain on the contraction curve of the m. tibialis anticus showing an increase in the contraction height followed by a decrease.

Graph 7. The effect of blocking the circulation to the lower limb on the contractions of the m. tibialis anticus. *A*, clamped abdominal aorta. *B*, stimulated sympathetic chain. *C*, removed clamp on aorta.

these results of sympathetic stimulation were due to the blood supply or whether they were of the type described by Orbeli for the bloodless muscles of frogs. It was found that, when the blood supply was withdrawn by clamping the abdominal aorta, the height of the muscle contractions decreased rapidly to almost complete fatigue within a few minutes. If the circulation was restored, the contractions returned gradually to their original height. Stimulation of the sympathetic nerve fibers while the muscle was deprived of its circulation did not cause either an increase or a decrease in the contractions (graph 7). The majority of the records, however, showed that the contractions did not fall off so rapidly when the sympathetic chain was stimulated. These facts indicated that the blood supply was, at least in part, responsible for the results of sympathetic

stimulation. Experiments were, therefore, undertaken to determine by what physiological mechanism sympathetic stimulation produced, first, the decrease and, second, the increase in the muscle contractions.

It seemed likely that the decrease in contraction height, which resulted from stimulation of the sympathetic fibers, was due to the presence of vasoconstrictor fibers in the sympathetic chain. In order to prove this, it was necessary to show that vasocontriction resulted from sympathetic stimulation, and that a decreased blood supply could cause a fall in the muscle contractions. Plethysmograph records, therefore, were taken of the volume of the leg below the knee. As the skin was always removed, any change in volume resulting from sympathetic stimulation was due to changes in the blood supply to the contracting muscles. Stimulation of the sympathetic nerve fibers, whether rapid or slow, always caused a de-



Graph 8. Plethysmograph record showing the effect of stimulating the abdominal sympathetic chain on the volume of the muscles of the lower limb while they are contracting.

crease in limb volume which proved that vasoconstriction occurred. The results were the same whether the muscles were at rest or contracting, and whether they were fresh or fatigued. That vasodilatation never resulted from sympathetic stimulation under the conditions of these experiments was doubtless due to the fact that the muscle vessels were already greatly dilated due to cutting the sympathetic chain, which destroyed the tonic vasoconstrictor impulses, and perhaps to the local action of the metabolic products formed during the contractions. This was evident from the plethysmograph records. When the sympathetic chain was cut, the plethysmograph record showed a marked increase in limb volume. When the muscle was stimulated to contract, the volume dropped sharply with the beginning of the contractions, as the blood was squeezed out of the

muscle; but while the muscle continued to contract, the volume gradually increased, soon becoming greater than the resting volume, thus indicating a local vasodilatation due to the products of metabolism. The limb volume continued to increase for a short while after the muscle ceased to contract and then remained constant for a long period. If the sympathetic chain was stimulated at any time during the record, the limb volume immediately dropped and remained at that lower level until cessation of the sympathetic stimulation (graph 8). If the sciatic nerve was cut, the vasoconstrictor effect disappeared at once, as the impulses could not reach the blood vessels in the contracting muscles.

Sympathetic stimulation, therefore, caused vasoconstriction which decreased the volume of blood supplied to the muscles. That a diminished blood supply caused the contractions to decrease in height was shown by the experiments in which the blood supply was temporarily blocked (graph 7). These two facts together prove that the decrease in contraction height, which follows sympathetic stimulation, is due to stimulation of the vasoconstrictor fibers in the sympathetic chain.

To determine the cause of the increase with sympathetic stimulation was a more difficult problem. Since the decrease in contraction height was due to vasoconstriction, the logical deduction was that an increase in blood supply was the cause of the increase in the contractions. This could result either from vasodilatation in the muscles, due to stimulation of vasodilator fibers in the sympathetic chain, or to vasoconstriction in the lower abdominal organs, thereby diverting more blood into the contracting muscles. There was no evidence, however, that an increase in the blood supply ever resulted from sympathetic stimulation under the conditions of these experiments. The plethysmograph records always showed a decrease in volume following sympathetic stimulation, even in those experiments where the previously recorded contractions had shown an increase. Moreover, in one experiment, where a plethysmograph tracing was made while the muscle contractions were being recorded, the increase in contraction height appeared simultaneously with a decrease in volume. Therefore, the increase in the contractions which resulted from stimulation of the sympathetic fibers was not due to an increase in the blood supply.

Because of the beneficial effect which adrenalin is known to have on the height of contracting muscles, it seemed quite possible that this substance might be produced as a result of sympathetic stimulation and be carried by the blood to the muscles, thus affecting the improvement in the contractions. However, in 4 out of 8 experiments, in which the adrenal glands were removed or the adrenal veins ligated, sympathetic stimulation still caused an increase in the contractions. The adrenal glands were, therefore, not entirely, if at all, responsible for the increased contractions.

The possibility existed, however, that sympathetic stimulation might

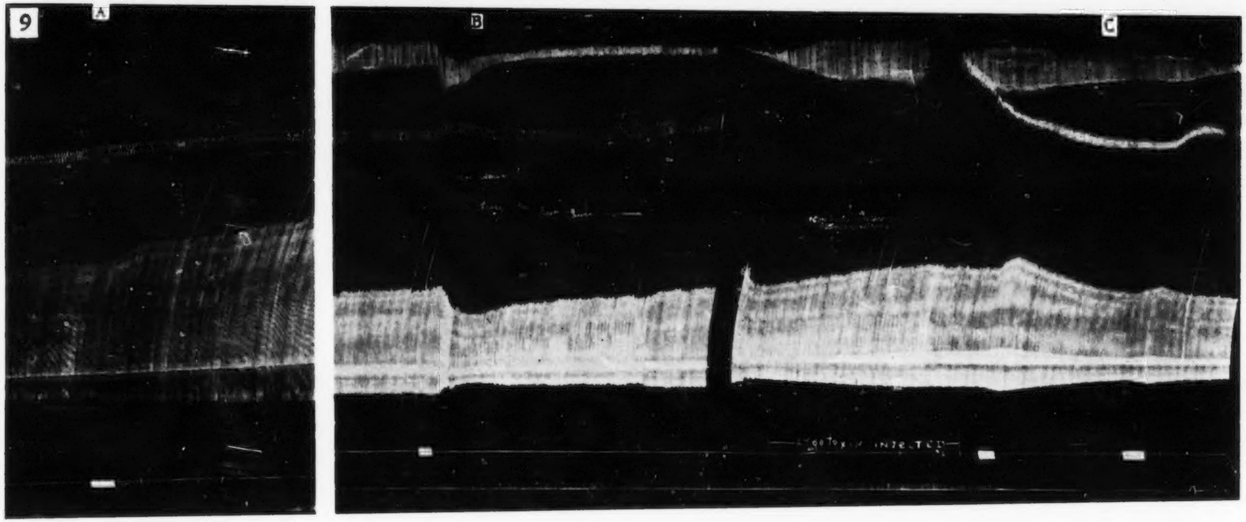
cause the formation of adrenalin in the extra adrenal chromaphil tissue which lies along the abdominal aorta, and which is known to be closely associated with the sympathetic chain. Since it was impossible to remove all the extra adrenal chromaphil tissue it was necessary to supply the contracting muscles with blood from an outside source so that no chemical substance, formed as a result of sympathetic stimulation, could reach the muscles. The remainder of the experiments were, therefore, performed with a cross circulation to the contracting muscles from a donor cat.

B. Cross circulation experiments. There were 9 successful experiments in which the blood to the contracting muscles was supplied by such a cross circulation. As the only connections between the lower leg and the body of the cat were the nerve fibers and as the skin was removed from the leg, any results of stimulation were due to nerve impulses to the contracting muscles or their blood vessels. The effect of sympathetic stimulation on the contraction curve was studied exactly as in the experiments with intact circulation, with similar results.

Sympathetic stimulation produced an increase in contraction height in 2 of these experiments, a decrease in 1, both an increase and decrease in 4, and no effect throughout 2 experiments and at times in the others. The increase was, therefore, evident in 6 experiments.

Since the blood to the contracting muscles was supplied from a second cat, no chemical substance such as adrenalin, produced as a result of sympathetic stimulation, could reach the contracting muscles. There were, therefore, only two possible explanations of the results of sympathetic stimulation under these conditions: either they were due 1, to stimulation of the vasomotor nerves, or 2, to stimulation of some unknown fibers in the sympathetic chain which produced these results apart from changes in the blood supply.

The plethysmograph experiments described above for the muscle with its normal circulation showed that the depressing effect of sympathetic stimulation was due to vasoconstriction, and that the increase was not due to vasodilatation. To test this conclusion under these conditions of cross circulation, a tracing of the volume of the contracting muscles was made simultaneously with the contraction curve throughout one experiment. At the same time a blood pressure record was taken from the femoral artery of the donor cat. As in the above experiments, sympathetic stimulation always caused a decrease in volume of the contracting muscles, indicative of vasoconstriction. The contractions in some cases showed a marked improvement simultaneously with this decrease in volume, as shown in graph 9A, and, at other times in the same experiment, a decrease, as shown by graph 9B. In those cases where the result of sympathetic stimulation was a decrease in the contractions, the blood pressure always increased.



A
Showing increase in contraction height with sympathetic stimulation and simultaneous decrease in blood volume of contracting muscles.

B
Showing decrease in contraction height with sympathetic stimulation and simultaneous decrease in blood volume of contracting muscles.

C
Showing increase in contraction height with sympathetic stimulation and no change in blood volume, following intravenous injection of ergotoxin.

Upper record.—Plethysmograph tracing of muscle volume.
Second record—Blood pressure in donor cat.
Third record —Contraction curve of m. tibialis anticus.
Fourth record—Sympathetic stimulation in experimental cat.
Fifth record —Time in seconds.

Graph 9. Cross circulation

An immediate rise in the blood pressure of the donor cat with sympathetic stimulation in the experimental cat could only be due to vasoconstriction in the muscles of the lower limb which were supplied by the cross circulation. Since sympathetic stimulation produced a fall in limb volume simultaneously with a rise in arterial pressure and a decrease in the muscle contractions, there was no doubt that marked vasoconstriction occurred in the contracting muscles, and that this was the cause of the decrease in the contractions. The increase in the contractions, when it occurred could, however, not be due to vasodilatation.

In another experiment the same conclusion was reached by counting the number of drops which flowed from the venous cannula before, during, and after a short period of sympathetic stimulation while the muscle was contracting as usual. The venous outflow decreased rapidly following sympathetic stimulation indicating vasoconstriction, while at the same time the muscle contractions showed an improvement.

The increase in the contraction height with sympathetic stimulation was, therefore, not due to a change in blood volume or rate of flow. It was also shown that a rise in blood pressure was not the cause of the improved contractions. Stimulation of the sympathetic fibers, when it resulted in an increase in the contractions, either produced no change in the blood pressure or a very slight rise. If the blood pressure in the donor cat was raised slightly, by stimulating the sympathetic chain in the donor cat, the contractions did not increase. Therefore, the increase in contractions was not due to an increase in volume, flow or pressure of the blood in the contracting muscles.

There must be, therefore, two types of fibers which were being stimulated in the sympathetic chain: 1, vasoconstrictor fibers, stimulation of which produced a decrease in blood supply and a fall in the height of the contractions, and 2, fibers which, when stimulated, caused an increase in the contractions, or, expressed differently, a temporary reduction in the fatigue. The effect, whether an increase or decrease, produced on the contractions of the muscle by stimulating the sympathetic chain was, therefore, the result of these two opposing reactions.

To further test this theory in one experiment where both an increase and a decrease were obtained at different times during the curve, it was decided to paralyze the vasoconstrictor fibers by intravenous injection of ergotoxin. This was given in the form of Gynergen, 3 mgm. per kilogram of body weight. Immediately following this, and throughout the remainder of the experiment, stimulation of the sympathetic nerve fibers always produced a rise in the contractions with no change in limb volume (graph 9C), although for ten times preceding the injection of ergotoxin only a decrease in the contractions had been obtained.

These experiments, then, confirmed, in cats, the results of Orbeli's

experiments in frogs, namely, that there is a type of nerve fiber in the sympathetic chain, which, when stimulated, produces an increase in the contractions of skeletal muscle. The experiments, however, throw no light on the mechanism by which this improvement is brought about.

Apart from the principal results of these experiments, there were two other points of interest which may be noted. In studying the cause of the rise in contractions following sympathetic stimulation, some experiments were made to determine if there actually was some substance produced by sympathetic stimulation and carried by the blood stream to the muscles, thus causing an improvement in contractions in addition to that which was due to the nerve impulses. This could only occur in those experiments where the circulation was intact. To test this it was necessary to cut off from the muscles all nerve impulses leaving only the blood supply as the effective agent. This was accomplished by cutting the sciatic nerve in eight of the experiments after the normal contraction records and the results of sympathetic stimulation had been obtained. The stimulating electrodes were moved from the anterior roots to the peripheral end of the severed sciatic nerve and the muscle contractions resumed by stimulating at this point. The strength and speed of the stimulation used was always below the threshold for the sympathetic fibers in the sciatic nerve. In every case following section of the sciatic nerve, which prevented any nerve impulses, arising from stimulation of the sympathetic, from reaching the muscle, sympathetic stimulation produced a slight increase in the contraction height, no matter whether there had previously been an increase or decrease before section of the sciatic nerve. This was also true if the contralateral sympathetic chain was stimulated. The increase was, however, never as marked as when the nerves were intact.

These results, however, could not be repeated in two experiments under slightly different conditions. In these animals the anterior roots were not dissected, the electrodes being placed at once on the sciatic nerve peripheral to its section. By not dissecting the spinal cord, the blood pressure remained higher and the muscles contracted more vigorously. Stimulation of the sympathetic chain under these conditions did not result in any increase in contraction height.

From these experiments it appeared that, if the animal was in a rather poor condition, sympathetic stimulation caused some slight improvement through the blood supply. This would indicate that some chemical substance, such as adrenalin, was being supplied by the blood. There is, however, one other possible explanation of this increase in contractions. Sympathetic stimulation caused a rise in blood pressure after cutting the sciatic, thus indicating vasoconstriction above the section of the sciatic nerve. By constriction of the blood vessels in the upper leg muscles or the lower abdominal organs, more blood might be diverted into the relaxed

vessels of the lower leg. This should result in an increase in the volume of the lower limb. A very slight increase in volume was found twice to occur with sympathetic stimulation after cutting the sciatic nerve.

Again to test this idea of some chemical substance being carried by the blood, an experiment was performed while the muscles were on cross circulation from a donor cat. Stimulation of the sympathetic chain in the donor cat, exactly as in the experimental cat, should bring about an improvement in the muscle response in the experimental cat if some chemical substance was produced by sympathetic stimulation and carried by the blood. There was, however, no evidence of a rise in the height of contractions when the sympathetic chain in the donor cat was stimulated.

A second point of interest was that after the administration of ergotoxin sympathetic stimulation, although causing no change in the volume of the leg, produced a rise in blood pressure in the donor cat, which appeared after a long delayed latent period of about 30 seconds. Since there was no vasoconstriction in the leg muscles, the only apparent explanation of the rise in blood pressure is that some substance was formed in the contracting muscles by sympathetic stimulation, which was carried by the blood to the donor cat, and there in some way caused a rise in pressure.

CONCLUSIONS

When the muscle tibialis anticus, in cats with a normal circulation, is contracting as a result of a series of single induction shocks applied to the anterior spinal roots, superadded stimulation of the abdominal sympathetic chain may cause either an increase or decrease in the height of the contractions. The decrease is due to vasoconstriction. The increase is not due to an increase in blood supply nor to any substance which may be carried by the blood, such as adrenalin, but must be due to stimulation of nerve fibers in the sympathetic chain other than the vasomotors, the impulses from which affect the muscle in some unknown manner, producing an increase in contraction height. When the vasoconstrictor fibers are paralyzed by the use of ergotoxin, stimulation of the sympathetic chain causes only an increase in the series of contractions.

The experiments, therefore, confirm, in mammals with normal circulation, the results of Orbeli's experiments on the bloodless muscles of frogs.

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THE POTENCY OF BLOOD SERUM OF MARES IN PROGRESSIVE STAGES OF PREGNANCY IN EFFECTING THE SEXUAL MATURITY OF THE IMMATURE RAT

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The presence of an ovarian hormone also termed folliculin and oestrin in the blood of non-pregnant sexually mature animals was first noted by Frank and co-workers (1925) and Loewe (1925) working independently. Fels (1926) found this substance to be present in increased amounts in gravid blood and this was later confirmed by Aschheim and Zondek (1928). Smith and Engle (1927) and Zondek and Aschheim (1927) working independently of each other demonstrated the anterior pituitary gonadal relationship. Since the publication of the work of Aschheim and Zondek (1928), in which they demonstrated the presence of folliculin and the sex maturing hormone of the anterior hypophysis in the blood serum and urine of pregnant women we have been interested in the concentration of these hormones in the blood of domestic animals. The present data are based on our studies in the mare. We have access to these animals so that they may be studied through all stages of pregnancy. We are concerned in this article with a study of the blood serum of non-pregnant mares and mares in progressive stages of pregnancy as regards its potency in effecting the sexual maturity of the immature rat. Further we are interested in the type of response elicited by serum taken at various stages of pregnancy and by the injection of different size doses from a single sample of blood.

It was soon found that the blood serum of mares in certain stages of pregnancy was effective in stimulating the immature genital system of the rat. The injection of the serum in a single dose as high as 10 cc. or a dose as high as 5 cc. repeated six times within a two day period, into immature rats, or a correspondingly large dose into white mice could be carried out without producing any deleterious effects in these animals.

METHODS. Exact breeding dates were available of all the mares used in our experiments Blood was drawn at definite intervals after breeding by tapping the jugular vein with a hypodermic needle. One to two hundred cubic centimeters of blood were drawn in each case into a sterile Erlenmeyer flask of 250 cc. capacity. After clotting had taken place the clot was separated from the sides of the flask by means of a sterile glass rod

and the flask placed in the refrigerator over night to allow the serum to separate. The serum was then drawn into a sterile syringe and injected subcutaneously in the abdominal region of the test animals after treating the site of injection with cotton saturated in 50 per cent alcohol.

In this work we established the normal genital tract of rats 26, 27 and 28 days old by killing them at that age and weighing their genital tracts. Abundant evidence exists that these animals never come into oestrum at this age under normal conditions.

Test animals. The test animals consisted of rats and mice either of which are satisfactory for the purpose. In our work a larger number of rats were used because they were available. In general the animals were used at weaning time (21 days). However, it was found entirely practical to vary the age from 18 to 23 days.

Ninety-six hours from the time of the first injection smears were taken, the animals weighed and killed with chloroform. The abdominal cavity was opened and the uterus severed from the vagina with scissors and removed with the ovaries intact. The uterus and ovaries were weighed together after which the ovaries and oviducts with bursae were separated from the horns of the uterus and weighed separately. The excess fat was removed previous to weighing without the use of binoculars. If one wishes merely to determine the presence of the factor or factors effecting sexual maturity in the blood this crude method is satisfactory but if it is desired to study the effect of varying doses it cannot be used. In such cases the oviducts and bursae were removed under the binoculars because this gave us a more accurate means of comparing the ovaries of the test animals with those of the controls.

After weighing, the tissues were placed in Bouin's solution and a large number of the ovaries were sectioned serially. In some cases control animals were used in this work which were the same age as the injected animals, but were not in all cases litter mates.

Positive results in the test animals were in some cases confirmed with a later diagnosis of pregnancy in the mares by means of manual examination of the uterus per rectum. This is somewhat more difficult in the mare than in the cow because of greater pressure being exerted on the arm and a higher degree of tension of the rectal walls, rendering slight changes in size or tone of the uterus unrecognizable. However, between the third and fourth month of pregnancy the developing fetus can usually be felt by ballottement through the rectal and uterine walls and this is sound evidence on which to base a positive diagnosis.

On September 28th we definitely established the existence of pregnancy in the following mares by this method:

No. 3 at 115 days
No. 4 at 116 days

No. 9 at 119 days
No. 5 at 135 days
No. 1 at 148 days
No. 2 at 156 days
No. 28 at 194 days
No. 7 at 213 days

EXPERIMENTAL WORK. *Blood serum from non-pregnant mares.* Serum from non-pregnant mares was taken at varying periods before the subsequent oestrum as follows: Mare 31, 5 and 10 days; mare 32, 5 and 16 days; mare 5, 3 days; mare 33, 12 days. The response of the genital system of the immature rat to six injections of 2 to 4 cc. of this serum was negative in all instances. It will be observed that we have not taken serum from mares at the time of active heat. However, the distribution of the cases with reference to the stage of the cycle is such as to warrant the statement that there is no substance in sufficient concentration in the serum throughout the greater portion of the cycle to elicit a response in the genital system of the immature rat when the serum is given in doses up to 24 cc.

Blood serum from pregnant mares. In table 1 is shown the reaction of the rats and mice to serum from mares taken from the 37th to the 222nd day of pregnancy. Although fifty six tests were made between the 10th and 222nd day of pregnancy only fourteen representative tests are included in the table.

The reactions were uniformly negative up to the 37th day of pregnancy. The time of the first reaction of the serum varied in different individuals from the 37th to the 42nd day of pregnancy. For example, mare 9 reacted on the 37th day of pregnancy and mares 10 and 5 on the 39th day. Mare 3 showed no reaction on the 26th, 33rd and 38th day but was positive on the 41st day. Mare 4 showed no reaction on the 27th, 34th and 39th day, but reacted on the 42nd day. The reaction of the rats to the serum taken at this early period in pregnancy could be observed macroscopically only in the uterus and vagina. The weight of the ovaries was comparable to that of control ovaries. However, when the ovaries were examined histologically after serial section, one or occasionally two follicles were usually found that were larger than follicles of control animals. This increase consisted chiefly of an enlargement of the follicular antrum. Ovaries from ten of the test animals which were comparable as regards weight to control ovaries were sectioned serially and compared with ovaries from four control animals. The test ovaries of three of these cases failed to show any variation in character from the controls. In the remaining instances there was an increase in the size of a single follicle from one to three hundred microns over the control which in no case exceeded five hundred microns. This increase in size of the follicular antrum was the only change observed. The ova of the largest follicles

TABLE 1

Data on reactions in immature rats and mice caused by serum from mares at various periods of pregnancy

NUMBER OF MARE	DAYS PREGNANT	FEMALE RATS OR MICE INJECTED	AGE OF RATS OR MICE IN DAYS WHEN KILLED	DOSE	NUMBER OF INJECTIONS	BODY WEIGHT WHEN KILLED	WEIGHT OF OVARIES AND UTERUS	WEIGHT OF OVARIES	RESULT		
									Ovaries	Uterus	Vagina
				cc.		*grams*	*mgm.*	*mgm.*			
9	37	B 390	26	2	6	57	180	30	−	+	Closed O
		W 422	26	5	1	58	60	30	−	−	Closed le
		W 394	26	7	1	43	60	20	−	−	Closed O
4	39	G 449	24	4	6	54			−	−	Closed le
		G 450	26	7	1	54	55	30	−	−	Closed le
10	39	W 222	24	2	6	41	165	40	+	+	Open Corn
		W 223	24	2	6	43	180	50	+	+	Open Corn
3	41	G 454	25	10	1	46	150	30	−	+	Closed Corn
		Mouse 61	25	.3	6	8	20		−	−	Closed le
		Mouse 62	25	.3	6	8	20		−	−	Closed le
11	41	B 462	26	4	6	53	320	190	+	+	Open Corn
4	42	G 456	26	4	6	48	170	30	−	+	Closed O
		G 457	26	7	1	46	195	30	−	+	Closed Corn
12	42	B 309	26	2	6	51	330	190	+	+	Open Corn
		B 310	26	2	6	57	330	170	+	+	Closed Corn
		B 311	26	5	1	51	215	70	+	+	Open Corn
15	54	W 268	27	2	6	63	420	250	+	+	Open Corn
		B 269	27	2	6	53	350	190	+	+	Open Corn
		B 270	27	5	1	62	410	205	+	+	Open Corn
30	62	G 288	23	2	6	49	320	160	+	+	Open Corn
		G 289	23	2	6	51	270	150	+	+	Open Corn
		W 293	22	5	1	39	260	120	+	+	Open Corn
7	75	W 119	27	2	6	68	485	305	+	+	Open Corn
		BH 11	27	3	6	65	450	220	+	+	Open O
		B 138	27	5	1	60	360	180	+	+	Open O
27	95	G 251	27	2	6	46	175	55	+	+	Closed O
		G 252	27	2	6	49	180	50	+	+	Closed O
		W 267	27	5	1	62	215	55	+	+	Closed O

TABLE 1—*Concluded*

NUMBER OF MARE	DAYS PREGNANT	FEMALE RATS OR MICE INJECTED	AGE OF RATS OR MICE IN DAYS WHEN KILLED	DOSE	NUMBER OF INJECTIONS	BODY WEIGHT WHEN KILLED	WEIGHT OF OVARIES AND UTERUS	WEIGHT OF OVARIES	RESULT		
									Ovaries	Uterus	Vagina
				cc.		*grams*	*mgm.*	*mgm.*			
7	117	W 231	26	2	6	49	230	50	+	+	Closed le
		W 235	27	2	6	42	110	30	−	+	Closed le
		G 161	27	4	6	48	195	50	+	+	Closed O
7	131	G 402	26	2	6	34	140	40	+	+	Open Corn
		W 405	26	4	6	56	180	55	+	+	Closed O-l
		G 407	26	5	1	54	225	30	−	+	Closed O
		B 408	26	7	1	48	210	40	+	+	Closed O-l
7	178	W 682	26	4	1	54	50	20	−	−	Closed le
		W 675	25	5	5	42	205	25	−	+	Closed Corn
		G 681	26	5	5	44	50	17	−	−	Closed Corn and leuc.
7	222	G 925	25	10	4	56	75	27	−	?	Closed Corn
		G 926	25	10	4	58	70	22	−	?	Closed Corn

Note: Weights of ovaries include oviducts and bursae.

Key for abbreviation in result column under vagina: Closed = vaginal closure membrane still unruptured. le = small epithelial cells and leucocytes. O = many small epithelial cells. O-l = many cells, both small epithelial and cornified. Corn = many cornified epithelial cells. Corn and leuc. = many cornified epithelial cells and leucocytes. The last four smears are all indicative of oestrum.

appeared in identical stages of development as those found in the controls, and it was impossible to distinguish any differences in the walls of the follicles in the test ovaries as compared with the controls.

After the 42nd day of pregnancy, the serum had a tremendous effect upon the size of the ovaries of the test animals, and changes were regularly induced in the vagina and uterus. The average weights of the ovaries from rats receiving serum taken at various stages of pregnancy are plotted in figure 1.

This figure shows that a maximal effect is produced in the ovaries between the 43rd and 80th day of pregnancy at about which time there is a gradual diminution in effect, appearing earlier in some cases than in others, until at about the 180th day of pregnancy when the test ovaries are again comparable in size to the ovaries of the controls. Reactions may still be obtained in the uterus and vagina of the immature rats until

the 222nd day and perhaps until the end of pregnancy, although at the 222nd day it was necessary to inject 30 cc. of serum in order to produce the effect.

Injection of varying size doses from the same sample of blood. Injections of serum from mares in different stages of pregnancy showed varying reactions in the ovaries of the injected rats. At certain stages medium doses produced maximal reactions. At this time we could vary the changes in the ovary from an enlargement of a single follicle to maximal reaction by simply varying the dose in different animals from the same sample of serum. For example, we took serum from a mare 78 days

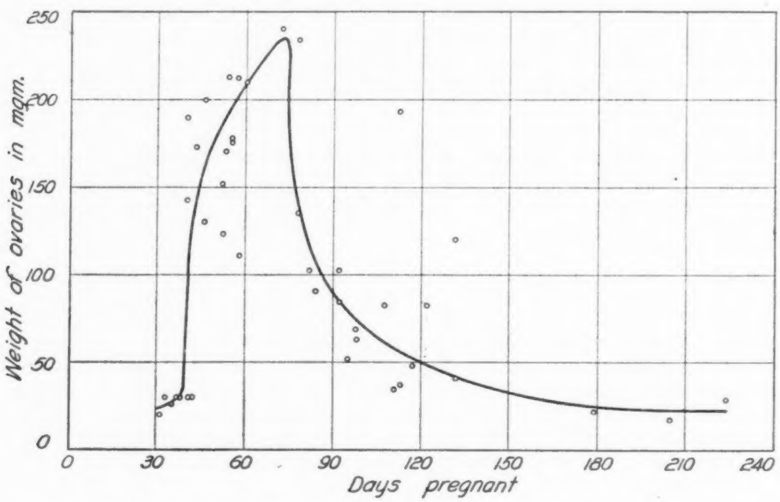

Fig. 1. Graph shows average weights of ovaries of rats injected with serum taken from mares at various stages of pregnancy. Weights include ovaries with oviducts and bursae attached.

pregnant and injected doses varying from $\frac{1}{500}$ cc. to 16 cc. into 73 rats. Eight controls were kept with this group, two of which received injections of physiological salt solution.

Animals receiving $\frac{1}{500}$ cc. of serum showed no reaction in the genital system. One out of four animals receiving $\frac{1}{300}$ cc. gave a definite reaction in the vagina and uterus, but showed no increase in the size of the ovary; two animals out of four receiving $\frac{1}{200}$ cc. and four animals out of four receiving $\frac{1}{100}$ cc. showed similar reactions. Serial sectioning of these ovaries revealed that in most instances the test ovaries could be differentiated from the control ovaries by an increase in size of a single follicle. In other words, the reaction produced was identical in character to that

obtained by injecting serum taken between the 37th and 42nd day of pregnancy. The ovaries of two out of four animals receiving $\frac{1}{20}$ cc. were definitely enlarged and ovaries from animals receiving larger doses were uniformly larger than ovaries from control animals.

Histological examination of the ovaries of rats receiving $\frac{1}{20}$ or $\frac{1}{10}$ of a cc. of the serum showed a marked increase in the size of a single follicle and usually of many follicles. The size of the follicles in many cases was double the size of the largest follicles of the control ovaries. These doses not only caused changes in the size of the follicles, but also produced further maturing signs in the follicles. The width of the stratum granulosum was greatly reduced, there being only two or three layers of granulosa cells in the wall of the follicle opposite the ovum. Occasionally a slight amount of luteinization could be discerned beneath the superficial granulosa layer. It was common to find both polar bodies formed from the ova in the ripe follicles, although the ova were still loosely attached to the stratum granulosum by the discus proligerus. The shedding of the corona radiata by these ripe ova was commonly observed and occasionally an ovum had divided into several segments. In instances in which the corona radiata was shed from the ovum, there often appeared the so called Call-Exner bodies in the granulosa cells of the discus proligerus. In one instance the rupturing of one follicle into an adjacent follicle was observed so that both ova were seen in the same follicle. The character of the ovaries just described simulated those from animals receiving serum from mares pregnant beyond the period of highest concentration of the hormone in their blood.

Ovaries from rats receiving 0.2 cc. showed luteinization of the larger follicles regularly and very often the remaining antrum was filled with red blood cells forming the blood points described by Aschheim and Zondek (1928). Increase of dosage up to 0.5 cc. resulted in the maturing of a larger number of follicles as well as increased luteinization and the more constant appearance of blood points within one or more follicles of the ovary. It is evident that luteinization and the formation of blood points result only from the larger doses of blood serum administered. Doses greater than 0.5 cc. failed in further stimulation of the ovarian response. Figure 2 shows that the average response of four animals as regards the weight of their ovaries was greater when 0.5 cc. was injected than at any other level. There is a wide divergence in the weights of ovaries from rats receiving similar size doses and it is impossible to attach any significance to this peak because of the relatively small number of animals used at each level. However, it would be of interest to know that an excess dose tended to inhibit the ovarian response.

Ovaries from animals receiving 0.5 cc. or more and killed ninety-six hours after the first injection did not show any signs of ovulation having

taken place and the ova could be found within the luteinized follicle in an altered stage as evidenced by loss of its corona radiata and changes in its staining properties. However, ovulation had taken place in some of the follicles when the animals were killed 120 hours after the first injection.

The results obtained by injecting 0.5 cc. or more of the serum were similar to those resulting from injections of serum taken from pregnant mares during the period of highest concentration of the hormone in their blood.

Assuming $\frac{1}{200}$ cc. to be the minimal dose, with which 50 per cent of the injected animals reacted, it took ten times this dose before definite

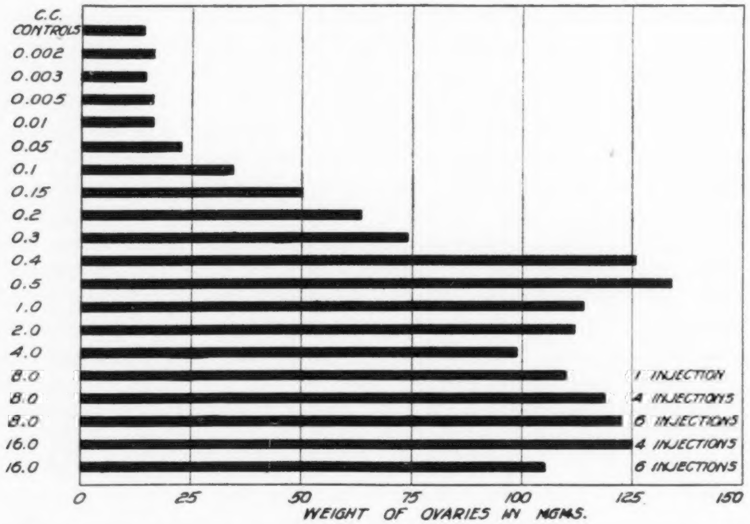

Fig. 2. Graph showing effect of varying doses of serum upon the size of the ovaries of immature rats. This blood was taken from mare 4 on the seventy-eighth day of pregnancy. Weights of ovaries represent average of the rats in each group. The oviducts and bursae were removed before weighing.

changes in the weight of the ovaries occurred. It took 80 to 100 times the minimal dose before maximal changes were produced in the ovary. This wide divergence between the minimal and maximal dose allows us to regulate carefully the response of the ovary that we wish to elicit.

We have not taken up the consideration of the relative concentration of the factor or factors causing the genital system response in different individuals during the period when maximal genital responses were obtained. However, we have evidence that there is considerable variation in the concentration in different individuals. For example, 0.5 cc. of serum

from mare 4 taken on the 112th day of pregnancy gave a greater increase in the size of the ovaries than 12 cc. of serum from mare 3 at the same stage of pregnancy.

In order to ascertain if folliculin as well as anterior hypophyseal hormone was present in pregnant mares normal female mature and immature rats were spayed and allowed to recover from the operation. Serum was taken from mare 4 on the 121st day of pregnancy. Four spayed rats were injected with 10 cc. per day of the serum and after 72 hours showed a reaction in the vagina by the presence of an oestrous smear. Four unspayed rats injected with 1 cc. of the serum and killed at 96 hours showed positive reactions in the ovaries. In this particular case 30 cc. of serum were required to produce the folliculin reaction whereas 1 cc. was sufficient to demonstrate the sex maturing hormone.

Serum taken at the 178th day of pregnancy from mare 7 did not produce any reaction in the ovaries of immature rats but did produce a reaction in the uteri and vaginae when 25 cc. were injected and from this point on until the 222nd day it was impossible to get a response in the ovaries. Tests have not been made later than the 222nd day of pregnancy. One mare was tested two weeks before foaling but only 12 cc. of serum were administered and it is possible that the negative reaction obtained at this time was because of the relatively small amount of serum injected in comparison with the amount that we know must be injected at the 178th to the 222nd day of pregnancy in order to produce a reaction.

During this work a total of 51 male rats and 7 male mice were injected with varying doses of serum that produced positive reactions in the females. With this number of injected animals there were maintained 35 controls. In all cases when the serum was at a high concentration there was an increase in the size of the testes of the injected rats in proportion to their body weights as compared to the controls. In many instances the increase in the size of the testes was small, never being more than double that of the controls. We have not taken into consideration a study of the accessory organs in the male. Due to the relatively small margin of difference between the injected animals and the controls, the males did not prove to be as satisfactory as the females in this work.

Discussion of results. It is significant to know that in the horse there are four typical reactions obtained varying in degree and in parts of the genital tract affected, depending upon the stage of pregnancy.

The earliest reaction from the 37th to the 42nd days of pregnancy is probably a reaction of the sex maturing hormone of the anterior hypophysis. Although changes in the ovary are slight the possibility of these changes being sufficient to induce changes in the uterus and vagina must be considered. We showed that we could induce similar changes by the injection of minute doses of serum known to contain the sex maturing

hormone of the anterior hypophysis in large quantities. This gives further indication of the reaction being one of the sex maturing hormone.

The reactions obtained from serum taken at the 43rd to about the 80th day of pregnancy are strikingly similar to the reactions obtained by Smith and Engle (1927) with implantation of hypophyseal tissue. Ovaries were produced in our cases at this period comparable in size to the largest ovaries produced by their implant method.

Between the 80th and 180th day there is a gradual diminution of the effect upon the ovary. At the later date the ovaries from animals giving clear cut reactions in the vagina and uterus were comparable to control ovaries both as regards their weight and their histological appearance.

Mare 4 gave both folliculin and a sex maturing reaction at the 111th day of pregnancy and we are of the opinion that the period between the 80th and 180th day is the period in which the sex maturing hormone disappears in the serum and folliculin appears.

The fourth period extending from the 180th day to later stages of pregnancy regularly shows reaction of the uterus and vagina alone. We have obtained this reaction up to the 222nd day of pregnancy but observations have not been continued past this point. Therefore we are probably getting a folliculin reaction alone at this time although we realize that the absolute test upon this point rests upon the chemical separation and subsequent testing of the separate fractions.

We are impressed with the correlation of the rise in potency of the serum with that of implantation of the fertilized ovum. The best data we have been able to find on implantation in the mare is that given by Kolster (1902). His investigations show that 28 days after breeding the amniotic sac has a size of 4.2 cm. and still is free in the uterus. After six to seven weeks it has a length of 12 to 14 cm. Its surface is covered with many recticular folds and the union between the sac and the uterine wall is well established. The weight of the embryo is sufficient at this time however for it to fall away from the uterine mucous membrane of its own weight if the uterus is opened. After nine to ten weeks the allanto-chorionic villi have extended into the sulci of the uterine mucosa. If we can accept these observations implantation in the mare occurs between the 40th and 70th days. Our observations show that the anterior hypophyseal hormone rises very rapidly in the blood to a maximum after its first appearance between the 37th and 42nd day. Further it remains in this relatively high concentration until about the 80th day. Keibel and Mall (1910) show in the human that implantation may take place as early as the seventh day and the Peters ovum is thought to have been fourteen days old and implanted for five days. The early implantation in women is accompanied with the appearance of the hormone in the urine at the end of the first week as shown by Aschheim and Zondek

(1928). That the much later appearance of this substance in the blood of the pregnant mare coincides with the later time of implantation is evidence to support the idea that this hormone may come to its highest concentration in the blood during the period of implantation on which very important part of intra-uterine life it may exercise some influence.

That the reaction obtained in immature rats by the injection of serum taken from mares 45 to 120 days after breeding are of great value in diagnosing pregnancy is beyond doubt. This is the period of time in which the diagnosis is of greatest value. During this period one is impressed with the uniform results obtained. A total of 184 test animals were used with positive reacting sera and only 13 of this number showed negative reactions. These 13 animals were all in tests of blood from two mares and received a single injection of 0.3 cc. or less. Therefore in diagnosis of pregnancy tests we think the use of two test animals twenty-one days of age is all that is necessary and from our work a single injection of 5 cc. gives a large margin of safety for the elicitation of the reaction.

We have some evidence that there is a reduction in the concentration of the hypophyseal hormone and an increase of folliculin late in pregnancy. Under such circumstances larger doses will be necessary to produce reactions in the test animals but other means of diagnosing pregnancy in the mare are available during this period. In case there should be sufficient folliculin in the blood stream at the time of active heat to elicit a reaction, which we have not eliminated, this still would not be a serious defect in the method because a test for the diagnosis of pregnancy would not be made at this time. When this test is made upon mares without breeding histories negative results would not eliminate a pregnancy of less than six weeks duration.

SUMMARY

The blood of 62 mares was tested for its potency in inducing the sexual maturity of immature rats. Serum from non-pregnant mares was negative in all instances as was the serum of mares up to the 37th day of pregnancy. The time of pregnancy at which the serum first gave a positive reaction varied in different individuals from the 37th to the 42nd day. Between the 43rd and 80th day of pregnancy the serum had the most marked effect upon the size of the ovaries of the test animals. At this time medium doses produced marked enlargement and extensive histological alterations of the ovaries of the experimental animals. At later stages of pregnancy the reactions to the injections of the serum were confined to changes in the uterus and vagina.

A correlation was observed to exist between the period of high concentration of anterior hypophyseal hormone in the serum and the time of implantation in the mare.

We were able with the same sample of serum given in varying doses to demonstrate a quantitative relationship between the amount injected and the reaction manifested in the ovary at definite stages of pregnancy.

The presence of the anterior hypophyseal sex maturing hormone in the circulating blood of pregnant mares may be used as a means of diagnosing pregnancy of six to seven weeks duration in these animals.

This reaction continues until about the 100th day of pregnancy. Later reactions are characterized by changes in the uterus and vagina only and are probably elicited by folliculin. Tests were not made beyond the 222nd day of pregnancy.

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THE INFLUENCE OF NUTRITION ON THE RESPONSE TO CERTAIN AMINO ACIDS

I. The Effect of Fasting

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In repeated experiments, when the same quantity of amino acid is administered orally to a dog which has been maintained under standard conditions as regards diet, exercise and environmental temperature, the response of the respiratory metabolism is surprisingly constant. This fact has been emphasized repeatedly by Lusk, who suggested that the constancy of the response entitles it to be classified with other physiologic constants, such as body temperature. When the amino acids are administered intravenously at the same rate of injection the nature of the response is equally constant. We have reported a series of experiments (Wilhelmj and Bollman, 1928) in which the amino acids, phenylalanine, alanine and glycine, were given in different quantities to different dogs and in which we found that the specific dynamic action for each millimole of amino acid deaminized was quite constant for the same amino acid, and the values obtained for alanine and glycine were approximately the same, whereas that for phenylalanine was nearly twice as great. In a larger series of experiments performed on a different group of animals we again obtained constant but lower values for the specific dynamic action of alanine and glycine. From time to time certain differences were observed in the behavior of the respiratory quotient after the intravenous administration of alanine and glycine. In seeking the cause for these variations we obtained evidence which seems to indicate quite clearly that the nutritional condition of the animal is a significant factor and that the specific dynamic action, as well as the respiratory quotient, following injection of the same amino acid, may be varied in the same animal by alteration of the nutritional status.

The experiments of Lusk have made it appear likely that the extra heat constituting the specific dynamic action of the amino acids, alanine or glycine, is probably not derived directly from the oxidation of the amino acid per se, since in completely phlorhizinized dogs he obtained a well

marked specific dynamic action in spite of the fact that approximately the entire energy content of the amino acid was eliminated in the urine as "extra" glucose and urea. These experiments seem to indicate that the extra heat is produced by the oxidation of materials already available in the body; if this is true, then it should be possible to alter the nature of the response by varying the nutritional condition of the animal.

Method of experiments. The method of indirect calorimetry employed has been described in detail by Boothby and Sandiford and adapted to animal use by Kitchen. Briefly, the method consists of an almost continuous collection of expired air in a gasometer for periods of ten or fifteen minutes, with an interval of three to five minutes between successive collections. Samples of the expired air are then collected over mercury and analyzed in duplicate with the Haldane gas analysis apparatus for the percentage of carbon dioxide and oxygen. Duplicate analyses are considered satisfactory when the percentages of carbon dioxide agree within 0.03 per cent and the oxygen within 0.04 per cent; if greater differences are obtained a third or even a fourth analysis is performed. The animals breathe outside air which passes over water and is warmed to approximately 25°C. The environmental temperature is maintained practically constant between 25° and 30°C., depending on individual differences in the animals.

In this series of experiments, the following procedures have been rigidly carried out: On the morning of the experiment the bladder was emptied with a sterile catheter and washed out with a constant measured volume of physiologic sodium chloride solution. Respiratory metabolism tests were then started, and during the next two hours at least six satisfactory tests were obtained. At the end of this period the bladder was again emptied and washed. The amino acids, alanine and glycine, were used in amounts equivalent to 0.1 gram of amino acid nitrogen for each kilogram of body weight (pre-fasting). This quantity of amino acid was dissolved in 50 cc. of distilled water, warmed approximately to body temperature, and injected intravenously into the saphenous vein at a constant rate so that ten minutes were always required for the injection. When phenylalanine was used, 4 grams were dissolved in 250 cc. of distilled water and injected in approximately forty minutes. The collection of expired air was started simultaneously with the injection of the amino acid and was continued for approximately four hours after the beginning of the injection. The expired air was collected for ten minutes, and the interval between successive collections was three minutes for the first hour and five minutes for the remaining three hours. Approximately two hours after the injection, the second sample of urine was taken, and the third was taken at the termination of the respiratory experiment. Catheterization was performed without removing the mask or disconnecting the animal from the gasometer. The animals were well trained and were not disturbed by the procedure.

One or two satisfactory experiments were first performed during a period in which the animals were on the standard diet used in the laboratory,[1] and were in a normal balanced condition of nutrition. The experiments were started approximately twenty-one hours after the last feeding. Under these standard conditions, the response to the injection of a given amino acid was found to be constant and the results obtained were considered as representing the normal response. After this, the animals were fasted for varying periods of time, and at frequent intervals during the fast the response to the same quantity of amino acid was determined in exactly the same manner as had been done while the animals were on the standard diet. Water was allowed ad libidum during the fasting period. After the period of fasting the animals were sometimes returned to the standard diet and the experiments were repeated at various times during realimentation.

Three female dogs were used. They had been used in metabolism studies for several years and were well trained and accustomed to the various experimental procedures. Previous to these studies they had been on the standard diet at the same time each day, and the weights had been practically constant for several months. Bilateral oöphorectomy had been done on all three animals approximately one year before the experiments were performed. Oöphorectomy is usually performed on all female animals to be used in metabolism studies over long periods of time since it has been observed that during estrus the animals are likely to be nervous, and irregular deviations in heat production will be manifested. After these experiments had been completed, the question arose concerning the possible influence of oöphorectomy on the results obtained. In order to be certain that this was not the factor responsible for the results, the experiments were repeated on a non-oöphorectomized female dog; the response of this animal was similar to that of the oöphorectomized animals.

In making the secondary calculations from our data, we have proceeded as follows: The oxygen consumption and carbon dioxide production, as determined in successive ten-minute tests, were expressed as liters for each hour and plotted as abscissas on coördinate paper with the time after injection of the amino acid plotted as ordinates; the total quantity of oxygen consumed and the carbon dioxide produced for four hours after the injection were then determined by means of a standardized planimeter.

Specimens of urine were collected, as described, in all instances except during the first fasting period with dog 1, in which glycine was used, and the first fasting period with dog 3, in which phenylalanine was used. In

[1] The diet for animals which are used in metabolic studies consists of 44 per cent ground fat-free beef heart, 44 per cent cracker meal, 8 per cent lard and 4 per cent bone ash. The basal requirement, with 50 per cent for maintenance, has usually been found ample to maintain the animal at a constant weight.

these two instances the heat production was calculated from the total oxygen consumption and observed respiratory quotients without regard to the protein metabolism and amino acid deaminized. In the remaining experiments in which the urine was collected, the total quantity of urea nitrogen excreted in the urine during the four-hour period after injection of the amino acid, minus the urea nitrogen of the basal period, was termed extra urea nitrogen and was considered as representing the amount of amino acid which was deaminized during the experiment. Calculated in a similar manner, the amount of extra amino acid nitrogen excreted during the four-hour period after injection was considered as representing the amount of amino acid which was excreted unchanged. The total nitrogen excreted during the four-hour period after injection, minus the extra urea and amino acid nitrogen, was considered as representing the protein metabolism. In the calculation of the nonprotein, nonamino acid respiratory quotient, the usual values were used for protein, whereas the values for the amino acids were those previously employed. From these data we have calculated the total heat production for the four-hour period after injection of the amino acid and the nonprotein respiratory quotient for the same period. The nonprotein respiratory quotient and the production of heat for the basal period were calculated on an average hourly basis. The basal heat production per hour when multiplied by the length of the experiment in hours, showed what the heat production would have been had amino acid not been given, and the difference between this value and the actual heat production after the injection represents the specific dynamic action for the four-hour period after the injection of amino acid.

THE RESPONSE OF NORMALLY NOURISHED ANIMALS TO THE INTRAVENOUS ADMINISTRATION OF ALANINE AND GLYCINE. In table 1 we have summarized the results of eleven experiments performed on three animals. These serve as controls for the studies during fasting reported in this paper and for the studies of fasting followed by carbohydrate diets reported in the second paper of this series. Certain features of the experiments shown in table 1 deserve special comment. The nonprotein respiratory quotients before injection were fairly constant and averaged 0.77, with maximal and minimal values of 0.83 and 0.74. In ten of the experiments there was a slight but definite rise in the respiratory quotient after injection of the amino acids so that the nonprotein, nonamino acid respiratory quotients for the four-hour periods following injection averaged 0.82, with maximal and minimal values of 0.86 and 0.78. This constancy of both the basal respiratory quotients and the quotients after injection, as well as the slight rise which occurs following injection, are dependent, as will be shown, on the fact that all three animals were receiving the same standard diet in equivalent amounts and were, therefore, in approximately the same nutritional balance. The total specific dynamic action for the four-hour periods was

TABLE 1

Summary of experiments performed while the animals were receiving the standard diet

Dog	Experiment	Date	Basal production of heat for each hour	Basal nonprotein respiratory quotient	Nonprotein respiratory quotient after injection	Total calories after injection	Length of experiment	Total specific dynamic action	Specific dynamic action for each millimole of amino acid deaminized	Specific dynamic action in per cent of: Calories given	Specific dynamic action in per cent of: Calories in amount deaminized	Per cent of amino acid deaminized	Comment
			calories				*hours*	*calories*					
1	1	11-11-27	15.4	0.77*	0.81*	70.7	4.16	6.5		56			5.56 grams of glycine; specimens of urine not collected
	2**	2-1-28	13.4	0.78	0.82	61.8	4.02	7.9	0.18	68	114	59	5.56 grams of glycine
	3**	2-3-28	13.6	0.79	0.81	62.3	4.12	6.3	0.19	54	117	46	5.56 grams of glycine
	4**	4-3-28	11.6	0.79	0.79	54.9	4.00	8.5	0.27	73	155	47	5.08 grams of glycine
	5**	4-6-28	12.0	0.76	0.82	59.3	4.05	10.7	0.22	92	127	72	5.08 grams of glycine
2	1**	11-28-27	17.6	0.79	0.85	75.4	4.00	5.0	0.25	17	78	21	8.54 grams of alanine
	2**	12-2-27	16.7	0.77	0.85	72.8	4.03	5.5	0.17	18	52	35	8.54 grams of alanine
	3**	3-6-28	17.6	0.83	0.84	77.8	4.08	6.0	0.16	20	51	39	8.54 grams of alanine
	4**	3-8-28	17.8	0.73	0.86	79.2	4.05	7.1	0.16	23	51	46	8.54 grams of alanine
3	1**	10-17-28	11.7	0.75	0.80	54.8	4.02	7.8	0.22	100	142	71	3.69 grams of glycine
	2**	10-19-28	12.1	0.74	0.78	56.0	4.02	7.4	0.19	95	123	79	3.69 grams of glycine
Average				0.77	0.82	Average for all			0.20				
Minimal				0.74	0.78	Average for alanine			0.19				
Maximal				0.83	0.86	Average for glycine			0.21				

* Observed respiratory quotient.

** With the exception of experiment 1 on dog 1 each pair of experiments served as a control for a fasting experiment or a fasting and carbohydrate diet experiment.

in general quite constant for the same animal even in experiments performed several months apart and between which a fasting or a carbohydrate diet experiment had been performed; the greatest variation is in experiment 5 with dog 1 in which the specific dynamic action was definitely elevated above the previous values. Wilhelmj and Bollman have given reasons for the belief that the specific dynamic action can best be correlated with the amino acid producing it by expressing the result as calories for each millimole of amino acid deaminized, and when the results of these experiments are expressed in this manner it is seen that the values were surprisingly constant in the three animals and that there was no essential difference between the values obtained for alanine and glycine; the average value for all experiments amounted to 0.20 calorie for each millimole of amino acid deaminized, whereas the average value for glycine was 0.21 calorie, and for alanine 0.19 calorie. The value of this method in comparing the specific dynamic action of amino acids in the same animal and to some extent in different animals may be well demonstrated in this series by comparing experiment 5 with dog 1, in which the total specific dynamic action for four hours following the injection of glycine amounted to 10.7 calories, and experiment 1 with dog 2, in which the total specific dynamic action after the injection of alanine amounted to 5 calories. There was a difference of more than 100 per cent in the total specific dynamic action in the two animals; however, when the results are expressed as calories for each millimole of amino acid deaminized, it is found that the values are essentially the same: 0.22 calorie in the former experiment and 0.25 calorie in the latter. Another example is seen in comparing experiments 4 and 5 with dog 1, in which it is seen that the percentages of amino acid deaminized amounted to 47 and 72 respectively, whereas the corresponding values for the specific dynamic action for each millimole deaminized were 0.27 and 0.22 calorie.

It is important to point out that these values, obtained for the specific dynamic action expressed as calorie for each millimole of amino acid deaminized are lower than the values obtained by Wilhelmj and Bollman, who obtained an average value of 0.35 calorie for each millimole of alanine deaminized and 0.45 calorie for glycine, in contrast to our values of 0.19 and 0.21 calorie respectively. Although there are many unknown and probably uncontrolled factors which may account for these differences, it is probable that these values may represent the upper and lower limits to be obtained on different animals, since figures identical with those of Wilhelmj and Bollman have recently been obtained by us on other animals. In table 1 we have also expressed the specific dynamic action in percentage of the physiologically available calories in the total amount of amino acid given and in the amount deaminized. These figures are also lower than the values given by Wilhelmj and Bollman. As pointed out by

them, this method of expression is open to certain criticism, but it is likely that the most significant figure is the specific dynamic action in percentage of the physiologically available calories in the amount of amino acid deaminized; in these experiments the average value for glycine was 130 per cent, whereas that for alanine was 58 per cent; this relative difference between alanine and glycine is comparable to the figures previously obtained.

THE RESPONSE OF FASTING ANIMALS TO THE INTRAVENOUS ADMINISTRATION OF AMINO ACIDS. *Alanine and glycine.* The changes in the response to the intravenous administration of alanine and glycine which occur during fasting, when compared to the response of the normally nourished animal, may be summarized briefly as follows: 1, an increase in the total specific dynamic action (the extra heat production above the existing basal value); 2, a decrease in the total heat produced during the four-hour period after the injection of the amino acid; 3, a much less marked rise, and in some cases even a lowering of the respiratory quotient, for the total four-hour period after the injection, as compared with the corresponding basal quotients; 4, a tendency for the amount of amino acid deaminized to become less, whereas the amount excreted unchanged shows relatively little change so that the total quantity of amino acid not accounted for by deaminization and excretion is usually increased, and 5, a tendency for the fasting animal to exhibit symptoms of a toxic nature (nausea and retching) at some period after injection of the amino acid; these toxic symptoms are practically never seen in normally nourished animals when the same quantity of amino acid is employed. These changes may now be considered individually and in more detail.

In the fifteen experiments performed on three fasting animals, it was found that the increase in the specific dynamic action ranged from 8 to 100 per cent or more above the values obtained on the same animals when they were on the standard diet and were in normal nutritional balance; experiments were not performed before the sixth day of fasting but this increase was noted from the sixth to the fourteenth days. The day on which the maximal increase occurred varied in different dogs and even in the same dog in different experiments; for example, during the first fasting period with dog 1, there was a progressive elevation from the sixth to the eleventh days, the increase amounting to 8 per cent on the sixth day, 35 per cent on the eighth day and 75 per cent on the eleventh day (fig. 1); during the second fasting period with the same dog, several months later, the maximal elevation of 86 per cent occurred on the sixth day, whereas the values on the eighth, tenth and thirteenth days amounted to 41, 32 and 38 per cent respectively (table 2). During the first fasting period with dog 2 the maximal increase of 109 per cent occurred on the ninth day, whereas the increases on the seventh, eleventh and fourteenth days were, respectively,

TABLE 2

Dog 1 second fasting period, 5.56 grams glycine

		BASAL PERIOD (FOR EACH HOUR)				AFTER INJECTION FOR 4 ± 0.06 HOURS							
Conditions	Date	Total oxygen	Protein nitrogen	Nonprotein respiratory quotient	Total calories	Total oxygen	Protein nitrogen	Extra urea nitrogen (glycine deaminized)	Nonprotein respiratory quotient	Total calories	Specific dynamic action	Specific dynamic action for each millimole glycine deaminized	Glycine unaccounted for during experiment
		liters	gram			liters	gram	grams					per cent
On standard diet	2- 1-28	2.830	0.62	0.78	13.4	12.919	0.221	0.611 (59.3)*	0.82	61.8	7.9	0.18	23
On standard diet	2- 3-28	2.865	0.72	0.79	13.6	13.164	0.501	0.470 (45.6)*	0.81	62.3	6.3	0.19	38
Sixth day of fasting	2-13-28	2.568	0.089	0.67	12.0	13.203	0.366	0.217 (21)*	0.70	61.4	13.2	0.83	75
Eighth day of fasting	2-15-28	2.577	0.062	0.68	12.0	12.541	0.341	0.370 (35.9)*	0.68	58.4	10.0	0.34	55
Tenth day of fasting	2-17-28	2.573	0.077	0.70	11.9	12.319	0.541	0.198 (19.2)*	0.67	57.0	9.4	0.67	74
Thirteenth day of fasting; glycine + 50 cc. of 25 per cent glucose	2-20-28	2.473	0.055	0.68	11.6	12.015	0.345	0.232 (22.5)*	0.74	56.2	9.8	0.58	71

* Per cent of glycine which was deaminized during time of respiratory experiment.

TABLE 3

Dog 2. First fasting period followed by realimentation, 8.54 grams alanine

		BASAL PERIOD (FOR EACH HOUR)				AFTER INJECTION FOR 4 ± 0.05 HOURS							
Conditions	Date	Total oxygen	Protein nitrogen	Nonprotein respiratory quotient	Total calories	Total oxygen	Protein nitrogen	Extra urea nitrogen (alanine deaminized)	Nonprotein respiratory quotient	Total calories	Specific dynamic action	Specific dynamic action for each millimole alanine deaminized	Per cent of alanine not accounted for during time of experiment
		liters	gram			liters	gram	grams					
On standard diet	11-28-27	3.686	0.042	0.79	17.6	15.632	0.152	0.279 (21)*	0.85	75.4	5.0	0.25	70
On standard diet	12-21-27	3.519	0.038	0.77	16.7	15.224	0.389	0.463 (35)*	0.85	72.8	5.5	0.17	53
Seventh day of fasting	12-12-27	3.254	0.065	0.69	15.2	14.563	0.102		0.71	68.2	7.4	**	92
Ninth day of fasting	12-14-27	2.894	0.071	0.70	13.5	14.112	0.288	0.091 (6.8)*	0.70	65.8	11.1	1.58	85
Eleventh day of fasting	12-16-27	2.878	0.043	0.70	13.4	13.424	0.464	0.237 (17.6)*	0.70	62.4	8.1	0.47	74
Fourteenth day of fasting	12-19-27	2.814	0.036	0.70	13.1	12.991	0.390	0.262 (19.5)*	0.68	60.4	8.0	0.47	71
Ninth day on standard diet	12-29-27	3.091	0.036	0.81	14.8	13.661	0.670	0.731 (54.5)*	0.98	65.4	5.8	0.11	32

* Per cent of alanine deaminized during time of experiment.

** No alanine deaminized during time of experiment.

40, 53 and 51 per cent above the average value obtained before fasting (table 3).

In order to demonstrate more conclusively that this increase in specific dynamic action is dependent on the nutritional status of the animal, two

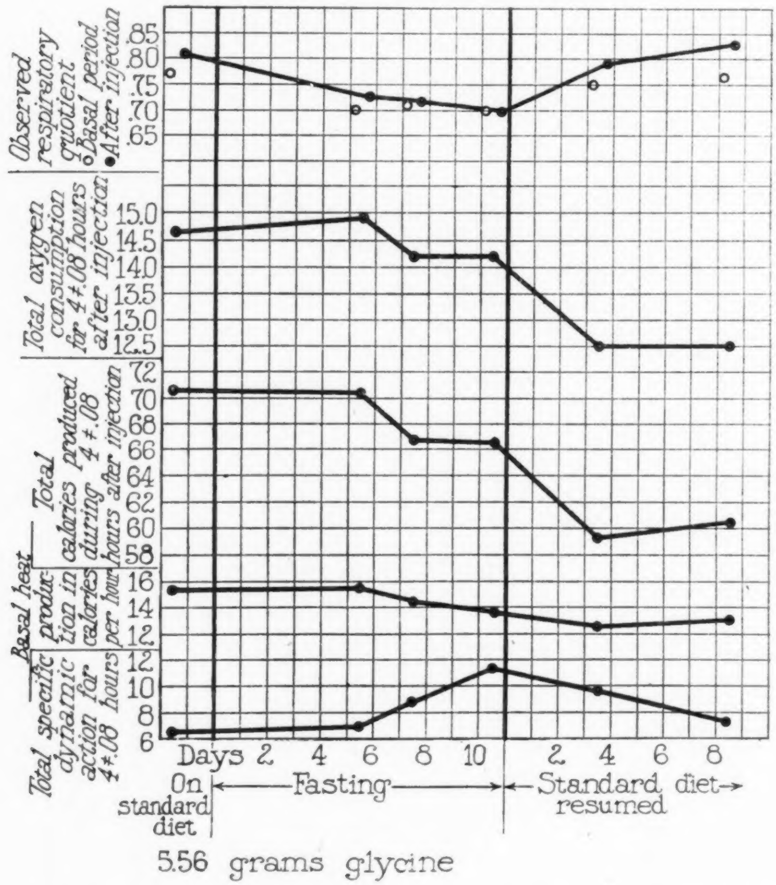

Fig. 1. First fasting period (dog 1). The response to 5.56 grams glycine during fasting and subsequent realimentation with the standard diet is shown.

of the dogs were placed on the standard diet after a fast of eleven and fourteen days, respectively, and the injections of amino acid were repeated. In these experiments there was a gradual decrease in the specific dynamic

action which reached the value obtained before the fast on the ninth day in both animals (fig. 1 and table 3). The gradual progressive nature of the decrease is clearly illustrated in the experiment with dog 1 (fig. 1).

The changes which occur in the specific dynamic action during fasting and subsequent realimentation are definite and marked, but the explanation of the mechanism producing them is rather difficult. In order to be certain that these changes are of direct metabolic significance it is necessary to be able to meet two criticisms which might be used to prove that the elevated specific dynamic action is a secondary phenomenon. First, as has been pointed out, fasting animals frequently become sick following the injection of alanine or glycine, and the increase in specific dynamic action might then be the result of muscular movements attending nausea and retching. Against this explanation, however, are the results obtained during the first fasting period with dog 2 in which there was no evidence of any such untoward reactions in any of the four experiments performed during a fourteen-day fast and in which the specific dynamic action was definitely elevated above the value obtained before the fast (table 3). During the first and second fasts dog 1 showed gross evidence of a reaction which was characterized by nausea and at times retching; great care was taken to watch for the slightest evidence of such a reaction, and the particular test during which it occurred was never used in calculating the final result; frequently also the test just before or just after a reaction was not used if the results were higher than those of entirely satisfactory tests just before or after the tests in question; in this way definite muscular movements were excluded from contributing to the increase in specific dynamic action. The second explanation which could be offered to show that the increased specific dynamic action is a secondary rather than a primary result, is based on the decrease in the basal production of heat which occurs during fasting. If the basal heat production decreases and the height of the curve of heat production after injection remains constant, then the increased specific dynamic action would represent an artifact dependent entirely on the decreased basal metabolism. An analysis of the data, however, shows that this is probably not the cause of the observed increase. First, the total heat production for the four hours after injection of the amino acid usually shows a progressive decrease during fasting which is in general parallel to the decrease in the basal heat production; in other words, the total heat production of the organism after an injection of amino acid decreases during fasting rather than remaining constant. Second, the basal heat production with dog 1 on the fourth day of realimentation was 1.3 calories per hour lower than the basal heat production on the eleventh day of fasting. Therefore, on this basis alone the specific dynamic action during a period of 4.03 hours could have been 5.2 calories higher on the fourth day of realimentation than on the eleventh day

of fasting; the actual figure, however, was 1.8 calories lower (fig. 1). Similarly, with dog 2 the basal heat production on the ninth day of realimentation was 2.4 calories per hour lower than the average value before the fast, so that during the four-hour experiment the specific dynamic action could have been nearly 10 calories greater; the actual value obtained, however, was practically the same as before fasting (table 3). Third, analysis of the relation between the drop in basal heat production and the specific dynamic action shows that in nearly all instances the increase in specific dynamic action which could have resulted from the drop in the basal heat production was much greater than the observed increase. Fourth, in the experiments with phenylalanine there was no increase in specific dynamic action during fasting in spite of the fact that there was a definite decrease in the basal heat production. These considerations seem sufficient to justify the conclusion that the increase in specific dynamic action of alanine and glycine which occurs during fasting is not the result of extraneous factors resulting from nausea and retching, nor can it be satisfactorily explained by the drop in the basal heat production which occurs during fasting.

The nonprotein respiratory quotients for the four-hour period after injection of the amino acids averaged 0.694, with maximal and minimal values of 0.71 and 0.65, in fifteen experiments performed on three dogs during fasting; in only four of the fifteen experiments were the quotients after injection below 0.70. The corresponding basal respiratory quotients averaged 0.69, with maximal and minimal values of 0.72 and 0.66. From this it is clear that during fasting there is no elevation of the respiratory quotient following injection of an amino acid. This should be compared with the results obtained with the same amino acids in the same animals when they were normally nourished, and there was a definite but slight elevation of the respiratory quotient, after injection. When the fasting animals were returned to the standard diet the basal respiratory quotients and the quotients after injection of the amino acids were found to return approximately to or above the value obtained before the fast. On the thirteenth day of the second period of fasting with dog 1, 12.5 grams of glucose were injected intravenously immediately after the injection of glycine; this caused the quotient for the total four-hour period to rise from 0.68 to 0.74, which is considerably above the quotient on the sixth, eighth and tenth days of fasting when glucose was not injected (table 2).

The total quantity of injected amino acid which can be accounted for by deaminization (extra urea nitrogen) and by excretion (extra amino acid nitrogen) during the four-hour period after injection was found, in general, to decrease during fasting. The percentage of amino acid given which remained unaccounted for by deaminization or by excretion approximately paralleled the increase in the specific dynamic action; the parallelism,

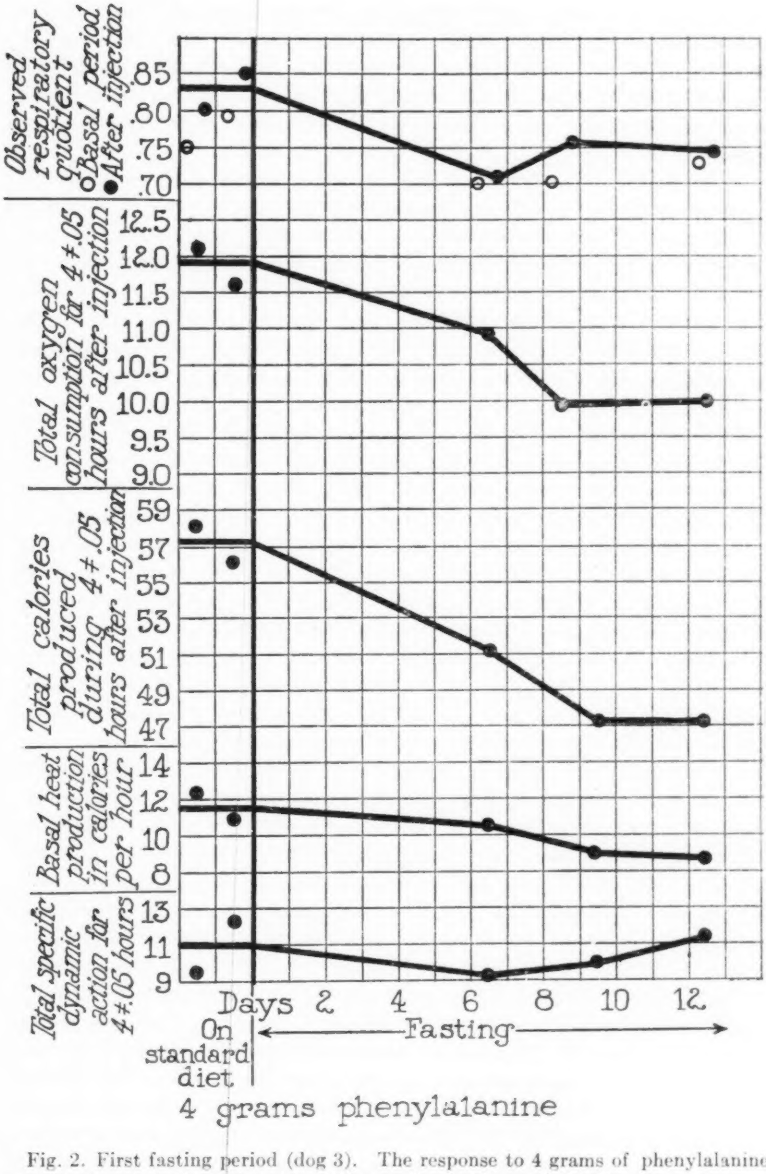

Fig. 2. First fasting period (dog 3). The response to 4 grams of phenylalanine with the standard diet and during a thirteen-day fasting period is shown.

however, was not exact since the greatest specific dynamic action did not necessarily occur on the day when the largest amount of amino acid remained unaccounted for during the four-hour period (table 3). When the animal was returned to the standard diet the amount of amino acid which was deaminized and excreted became greater than during fasting (table 3).

Phenylalanine. The results of fasting on the response to phenylalanine are shown in figure 2. It may be seen that the total heat production for the four-hour period after injection became progressively less during the thirteen days of fasting and that the basal heat production showed a decrease which is comparable to that observed in the experiments with alanine and glycine. The specific dynamic action, however, showed a slight tendency to decrease rather than to increase, but the changes were well within the limits of the values obtained while the animal was receiving the standard diet. From this it is clear that during fasting there is practically no change in the specific dynamic action of phenylalanine. Specimens of urine were not collected in this experiment, and the respiratory quotients were the observed quotients and not the nonprotein quotients.

COMMENT. The changes in the response to the intravenous administration of alanine and glycine which were found to occur in fasting animals seem to be of metabolic significance. The increase in the specific dynamic action (expressed as the extra calories of heat production above the prevailing basal level), the lower total heat production during the four-hour period after injection, the failure of the respiratory quotients to rise above the fasting basal value, the evidences of a toxic reaction and the smaller quantity of amino acid accounted for by deaminization and excretion, should probably be considered as related phenomena.

Several hypotheses might be advanced to explain the increase in specific dynamic action during fasting, and at least three of them seem to be worthy of some consideration. They are that: 1, it represents the extra heat required in synthetic processes in which the simple amino acids are built into more complex nitrogen-containing compounds; 2, it is due to the catalytic-like action of an intermediate compound formed after deaminization of the amino acids which, by virtue of its specific chemical configuration, stimulates the cells to a higher level of metabolism; on this assumption the intermediate substance would be assumed to be rapidly destroyed or chemically changed in the body of well nourished animals but to accumulate in the fasted animal and thus be responsible for the increased specific dynamic action and the toxic symptoms, and 3, it may be the result of quantitative or qualitative changes in the process of conversion of the amino acids to glucose, thus also explaining the differences which we have noted in fasting animals between the action of alanine and glycine which are converted to glucose and phenylalanine which, according

to Dakin, does not form glucose. The third hypothesis is interesting in view of the calculations reported by Lusk at the XIII International Physiological Congress in which he showed that the energy involved in converting glycine and alanine to glucose would appear sufficient to account for their specific dynamic action.

As the evidence for and against these hypotheses is critically reviewed, it becomes obvious that none is as yet capable of direct proof and can only serve as a guide in the search for more conclusive data. The term specific dynamic action is generic, used to designate the elevation in heat production which follows the ingestion of the primary foodstuffs and certain of their cleavage products. It is likely, indeed highly probable, that the production of extra heat caused by the amino acids is not always the result of the same chemical processes taking place in the body, but that the type of chemical change giving rise to the specific dynamic action may change under certain conditions; for instance, in the well nourished animal the amino acid may represent an excess not needed in the body economy, while in the fasting dog it may represent a valuable material which the body conserves and uses.

An attempt has not been made to review the enormous literature on fasting, but there are a few articles which should be mentioned as being pertinent to the experiments reported. Plaut has presented evidence indicating that the specific dynamic action of a protein meal is much less in cases of pituitary obesity than in normal subjects. Jaquet and Svenson, and Staehelin presented evidence which they interpreted as showing that obese subjects have less reaction to food than normal subjects. DuBois, on the other hand, recently reported that the specific dynamic action of protein in obese subjects is not essentially different from that in normal subjects. Mason showed that in cases of undernutrition an abnormal specific dynamic action from one or more of the primary foodstuffs is often manifested, and that the state of nutrition often improves when this foodstuff is limited in the diet. In his cases of undernutrition, the total response in heat production during a given period after the particular meal had been taken, was less than normal but the specific dynamic action was greater. Gibbons has compared the specific dynamic action of meat in a fat dog and in a thin dog of the same weight and found that it was considerably greater in the thin dog. McCann determined the effect of a protein meal on a man at the end of an eight-day fast and again after he had been on a normal diet for one week. In the first instance, the specific dynamic action for a three-hour period after the meal was 17.6 calories and in the latter it was 46.0 calories; the total heat produced for three hours after the meal was practically the same in both conditions, amounting to 238.7 and 236.5 calories, respectively. While his results as regards the specific dynamic action are therefore the reverse of what we found in dogs,

using alanine and glycine, an analysis of his data shows that his results can be explained by the change in the basal level of heat production which amounted to 73.7 calories per hour on the eighth day of fasting and 63.5 calories per hour after one week on a normal diet. At the end of the eight-day fast he observed nonprotein respiratory quotients of 0.693 and 0.686 after the meal of meat, and suggested that they are produced by the same mechanism as in diabetes, except that the glucose, instead of being excreted in the urine, was stored as glycogen. Honda recently determined the specific dynamic action of meat in rats which had previously been given food high in fat, high in carbohydrate, or consisting of meat and peptone while being injected with phlorhizin. In the animals fed on fat he found a very low specific dynamic action; in the animals fed on meat and peptone while they were being injected with phlorhizin there was a very high specific dynamic action. The animals fed carbohydrate showed a specific dynamic action greater than the first group and less than the second group. Honda suggested that these changes are associated with changes in the function of the liver resulting from the diets. Jahn and Strössenruther recently showed that the specific dynamic action can be influenced by the previous diet.

SUMMARY

The response to the intravenous administration of the amino acids, alanine and glycine, is constant in well nourished animals which have been maintained on a constant standard diet. The uniformity of the response is displayed in the respiratory quotient, the total specific dynamic action, and the specific dynamic action for each millimole of amino acid deaminized. In a series of eleven experiments performed on three well nourished animals, the average respiratory quotient for the four-hour period after injection of the amino acid averaged 0.82, with a maximum of 0.86 and a minimum of 0.78 in different experiments. The corresponding basal respiratory quotients averaged 0.77, with maximal and minimal values of 0.86 and 0.78. There was therefore a slight but definite rise in quotient following administration of the amino acids. The specific dynamic action for each millimole of amino acid deaminized averaged 0.20, with maximal and minimal values of 0.16 and 0.27 calorie.

During fasting there are certain well marked alterations in the response to the amino acids, alanine and glycine. These changes consist of: 1, an increase in the total specific dynamic action; 2, a low respiratory quotient during the four-hour period after injection which averaged 0.69 in fifteen experiments on three fasting animals; the corresponding basal quotients likewise averaged 0.69, so that in general the quotient after injection of the amino acid is identical with the basal quotient; 3, a decrease in the total calories produced during the four-hour period after injection; 4, a decrease

in the amount of amino acid which can be accounted for by deaminization and excretion, and 5, evidence of a toxic reaction occurring some time after injection (from twenty-six minutes to one hour or more) in some but not all animals. If the animal is returned to the standard diet after approximately two weeks of fasting, there is a gradual return to normal in the nature of the response to the injection of alanine and glycine. The intravenous injection of glucose before or just after the injection of the amino acid in the fasting animal causes a slight rise in the respiratory quotient for the four-hour period after injection, but no significant alteration in the specific dynamic action, nor does it prevent the onset of toxic symptoms.

There is no change in the specific dynamic action of phenylalanine during fasting, but the respiratory quotient after injection is approximately as low as in the experiment with alanine and glycine.

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THE DISTRIBUTION OF BLOOD CALCIUM IN THE CIRCULATION OF LAYING HENS

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In the economy of the laying hen, calcium exists in the bone, mainly as tricalcium phosphate, and is excreted as carbonate in the egg shell. In developing the improved breeds of chickens, man has, for his own purposes, caused the metabolism of the laying hen to increase in speed and it is now necessary for the hen to approximate the 300 egg mark, annually, instead of the 20 to 30 eggs normal to the progenitor of the modern chicken. This means a greatly increased elaboration of protein and calcium carbonate, there being about 5.5 grams of calcium carbonate in the shell of a normal egg. Since there are no calcium or protein storage organs in the chicken, to meet the increased demand, it follows that these substances must be supplied in the daily food in appropriate form and quantity. The intake of food must supply, also, the calcium necessary for building tissues and assisting in the many biologic processes in the body. It is reasonable to believe that the ordinary food, such as grain, meat scrap, milk and vegetables, will supply the substances, including calcium, which are necessary for the processes normal to the life cycle of fowls, but that supplemental calcium carbonate is necessary, in order to satisfy the abnormal conditions imposed by man. It is a well established fact that the ingestion of calcium carbonate greatly increases the number of eggs produced by a hen.

Calcium compounds in the food pass into the glandular stomach, which contains, among other things, free hydrochloric acid and water. Here the calcium compounds, largely calcium carbonate and tricalcium phosphate, undergo some changes, but it seems impossible that the hydrochloric acid of the stomach is sufficient for the solution of these two water-insoluble compounds in such quantities as conditions demand; that is to say, for the absorption and secretion of approximately 5.5 grams of calcium carbonate every 24 hours for many successive days. In fact, the actual time of secretion of these 5.5 grams of calcium carbonate as egg shell is

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

estimated at not more than 16 hours. This calcium is delivered by the blood stream to the oviduct where it is secreted principally as the egg shell. The calcium in the blood is probably in the form of ionized calcium bi-carbonate, calcium phosphates, calcium chloride and possibly some calcium-protein complex. The calcium content of the blood of a laying hen has been shown to be greater than that of a non-laying hen.

The object of this investigation was to determine the distribution of blood calcium in the circulation of laying and non-laying hens as it was desired to know to what extent calcium was taken directly into the blood stream from the food in the intestines under these conditions.

TABLE 1

The calcium content of the serum of blood from the anterior mesenteric artery, the anterior mesenteric vein and the left ventricle of hens expressed in milligrams of calcium per 100 cc. of serum

HEN NUMBER	LAYING CONDITION	POSITION OF EGG	ARTERIAL BLOOD	VENOUS BLOOD	LEFT VENTRICLE BLOOD
1	Active	Egg partly formed in uterus	23.6	25.4	24.9
2	Active	Shell-less egg in isthmus	23.2	25.4	
3	Active	Shell-less egg in isthmus	12.9	14.5	
4	Active	Shell-less egg in isthmus	24.5	29.6	26.2
5	Active	Egg in uterus, shell partly formed	17.6	21.0	19.7
6	Active	Fully formed egg in uterus	17.6	18.8	
Average for the 6 hens			19.9	22.4	
Average for hens 1, 4 and 5			21.9	25.3	23.6
7	Inactive	Had not laid for 2 weeks	17.8	17.7	
8	Inactive	Not in active laying state	19.1	19.4	
9	Inactive	Moulting	11.3	11.2	
Average for 3 hens			16.1	16.1	

With this end in view we obtained a number of White Leghorns of the same age and stock, whose laying conditions were known and that had received the same feed which contained ample calcium carbonate.

After many trials, we found that chloral hydrate served most satisfactorily as a general anesthetic for chickens. The dosage used varied with conditions. One hundred and twenty-five cubic centimeters of a 1 per cent solution gave complete anesthesia to a 5 pound White Leghorn hen whose crop was empty, but 100 cc. of a 2 per cent solution was required, if the hen had recently consumed food. The dosages were given by means of a rubber bulb attached to a rubber catheter, directly into the crop. From 3 to 8 minutes were usually required for complete anesthesia. At this point a

three inch incision was made into the left lower abdomen and the intestines carefully lifted from the abdominal cavity. A 19 gauge hypodermic needle was introduced into the anterior mesenteric artery and about 10 cc. of blood obtained. The bleeding was arrested with hemostatic forceps and the intestines inverted, another needle inserted into the anterior mesenteric vein and about 10 cc. of venous blood obtained. In some instances when the heart was still beating vigorously a needle attached to a hypodermic syringe was introduced directly into the left ventricle and 10 cc. of blood withdrawn.

The blood, in small test tubes, was placed in an incubator at 37° for 1 hour and allowed to clot. At the expiration of this time, the clot was loosened from the glass by means of a platinum wire and the sample placed in an ice box for 12 hours. The clear serum was drained off and calcium determined in the separate samples according to Clark and Collip (1925). The results of these determinations are given in the table.

In 6 actively laying hens the average calcium content of the serum of blood obtained from the anterior mesenteric artery was 19.9 mgm. per 100 cc. and that of the blood from the anterior mesenteric vein of the same hens was 22.4. This shows that, expressed as averages, the blood coming from the intestines contained 2.5 mgm. per 100 cc. more calcium than the blood going to the intestines.

From 3 of these laying hens we were able to obtain samples of blood directly from the left ventricle of the heart. The average calcium content of the serum of blood obtained from the anterior mesenteric artery was 21.9, that from the anterior mesenteric vein was 25.3 and that from the left ventricle was 23.6 mgm. per 100 cc. of blood.

Post mortems showed that in each of these 6 hens there was an egg in the process of formation in the oviduct and chemical analysis showed that there was a large excess of calcium carbonate in the intestinal tract.

In three hens that were not in a laying condition the average calcium content of the serum of blood from the anterior mesenteric artery was 16.1 mgm. of calcium for 100 cc. and that obtained from the anterior mesenteric vein was the same. Post mortems showed atrophied oviducts which are normal for non-laying hens and there was an abundance of calcium carbonate in the intestinal tract.

From these results it will be seen that in non-laying hens the arterial blood going to and the venous blood coming from the intestines contain the same amount of calcium, which shows the circulation to be in a state of calcium equilibrium.

In actively laying hens the calcium content of the blood was higher than that in non-laying hens.

In actively laying hens the calcium content of the blood obtained from the anterior mesenteric vein is appreciably larger than that of blood

obtained from the anterior mesenteric artery and the blood from the left ventricle was intermediate between these values. In other words, the arterial blood going to the intestines containing calcium carbonate takes up calcium and passes through the veins to the heart where it is apparently diluted with blood which must contain a smaller quantity of calcium.

Repeated efforts have been made also to obtain samples of arterial and venous blood from the oviduct and legs of laying hens but in all cases the hens have died before this was possible. However, we are now developing a technique with which it is hoped to extend these investigations and throw further light on the process of transferring calcium in the intestines of laying hens to their oviducts and its deposition as egg shell.

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THE ACTION OF COMPRESSION ON THE CONTRACTION OF HEART MUSCLE[1]

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We have shown that when heart muscle or skeletal muscle is immersed in either liquid petrolatum or Ringer's solution and pressure is applied to these surrounding liquids, thereby transmitting the force uniformly to the tissue, there is a marked increase in the height of the contraction during the period of compression (1928a, b). Some of the previous work bearing immediately upon our experimental observations was reviewed in these articles, but it is of interest to observe further that the general subject of the influence of pressure on living tissue stimulated investigation very early. However, the pronounced effects of pressure on the contractility of muscle do not appear in any of the results reported by the original workers in this field.

The observations of Poiseuille (1835) are noteworthy, since he was the first apparently to show that pressures up to 8 atmospheres produce no observable change in the peripheral circulation, as seen in the transparent parts of the salamander and also of the frog. Regnard (1891) made records of the heart beat of the frog at 100, 200, and 300 atmospheres, and his curves exhibit a decrease in both amplitude and rate at these pressures, and complete standstill of the heart at 400 atmospheres. His failure to discover the augmented heart action which occurs at pressures around 100 atmospheres, as used in our work, may have been due to the fact that he did not make observations soon enough after the application of pressure.

We have not tested the action of pressures higher than 110 atmospheres, but the observations of Regnard have been confirmed by others, that exposure to a pressure of 400 atmospheres produces injurious effects. Regnard (1891) states that pressures of 400 to 500 atmospheres, applied to a frog's muscle that was suspended in water, caused swelling and rupture of the fibers, and Hill (1912), working with a reflex preparation of the frog, states that exposure to 400 atmospheres for 1 hour produces paralysis and that a structural examination reveals some disorganization of the muscle

[1] An abstract of part of the work reported in this paper was printed in the Proceedings of the XIIIth International Physiological Congress, This Journal, 1929, xc, 337.

material, with alterations of the myelin of the nerve fiber. On the other hand, the latter observer reports that subjecting the frog preparation to a pressure of 300 atmospheres for 2 hours produced no change in the reflexes and no evident effect upon the heart action. From some experiments that appear to be inadequately controlled, Henderson, Leland, and Means (1908) make the deduction that a pressure of 500 atmospheres, gradually applied and released, may be without effect on muscle contraction.

In the present experiments we are concerned mainly with the form and the time-course of the contraction of cardiac muscle exposed to compression. In the experiments previously reported we used an optical membrane manometer which was so arranged as to record the intraventricular pressure changes. The entire manometer with heart attached was contained in the compression chamber, a condition which might have introduced a confusing factor in the effect of pressure on the elastic membrane of the system. In order to obviate this possible objection a special torsion spring lever has been constructed which is patterned after the general principles employed by Fulton (1925a). The design of the mounting enables us to use either strips of the ventricle from the heart of the turtle or the entire ventricle. When the latter is employed one edge of the organ is placed in a clamp and the other fastened to the lever. In these experiments we have commonly used the entire ventricle, but strips appear to give equally good results. The compression chamber and the method of producing and controlling the pressure was the same as that previously described (1928a).

Results. In our previous experiments, in which the method of fluid displacement from the ventricle was used as a measure of contraction, we showed an average increase of over 90 per cent in the magnitude of the response of the muscle when subjected to compression. When the heart muscle acts upon a tension lever the augmenting effect of pressure is somewhat less, but there is a greater degree of constancy in the results of the individual experiments of the series. In the 18 experiments given in table 1 the average "pressure effect" was 41.8 per cent, using ventricle preparations with the tension method and pressures ranging from 960 to 1000 pounds per square inch. The difference between this figure and the one previously reported appears to be due to the difference in the type of preparation and the method of recording. With a rhythmic preparation as formerly used the greatest augmentation occurs immediately after the application of pressure and then the beats show a gradual diminution. Our measurements were made on the early cycles which showed maximal stimulation, but in the present series a quiescent preparation was used and the records were obtained by stimulating the heart at constant intervals of 1 minute.

We have attempted to obtain information concerning possible changes in the mechanism of contraction during periods of compression by analyzing the time course of the phases of developing and releasing of tension. The method employed consisted of taking about 3 records at 1 minute intervals as soon as the preparation was set up in the pressure chamber. This served as the control series. Then the compression was placed upon the liquid surrounding the heart and immediately another group of about 3 records was made for the pressure series.

TABLE 1

The influence of moderate pressure on the duration of the phases of contraction and relaxation of ventricular muscle

EXPERIMENT NUMBER	PRESSURE	STIMULATION (TENSION)	DURATION OF PHASES			
			Contraction		Relaxation	
			Control	Pressure	Control	Pressure
	pounds	*per cent*	*second*	*second*	*second*	*second*
19	980	46.7	1.12	1.12	1.01	1.22
20	980	38.9	0.84	0.86	0.82	1.09
21	980	78.6	1.05	1.13	0.54	0.55
25	1,000	37.8	1.22	1.30	0.50	0.58
28	1,000	27.8	1.13	1.12	1.22	1.24
30	980	49.0	1.40	1.40	0.57	0.70
31	980	54.5	1.78	1.77	0.65	0.74
33	980	20.4	1.87	1.93	1.67	1.73
34	990	34.4	1.52	1.48	1.62	1.62
35	1,000	41.4	1.24	1.28	1.23	1.33
36	980	33.3	1.04	1.03	1.06	1.11
38	1,000	44.4	1.09	1.04	1.59	1.83
39	980	38.9	1.05	1.08	1.10	1.19
41	960	36.2	0.99	0.99	1.01	1.09
42	960	20.0	1.17	1.20	1.35	1.38
43	980	72.2	0.74	0.85	0.78	0.87
45	980	28.3	1.46	1.44	1.23	1.34
46	1,000	40.0	1.50	1.47	1.17	1.25
Average..	985	41.8	1.23	1.25	1.06	1.16
			1.5%		9.43%	

In making measurements of contraction and relaxation times a difficulty arises in placing accurately on the tension curve the position where one phase ends and the other begins. This has been obviated to some extent by the fact that in many instances our tracings show a fairly sharp onset of the subsidence of tension which is suggestive of the "angle" that Fulton (1925b) describes for skeletal muscle. We assign no significance to this feature but employ it merely to give a point sufficiently

defined to permit of more accurate measurements of the two phases in the curves. A typical tension record from a ventricle preparation is given in figure 1 for the purpose of showing the abrupt beginning of the decline in tension. While great accuracy cannot be claimed for the measurements of the phases of the tension curve, there is no evident reason why errors should occur more frequently in the pressure series than in the controls, therefore we accept the average values as a very close approximation to the true time relations.

The results in table 1 show that, whereas the maximum tension under pressure was increased about 42 per cent, the total time required to develop this additional tension was increased only 1.5 per cent. Viewed in the light of the mechanics of contraction, this result denotes that a more

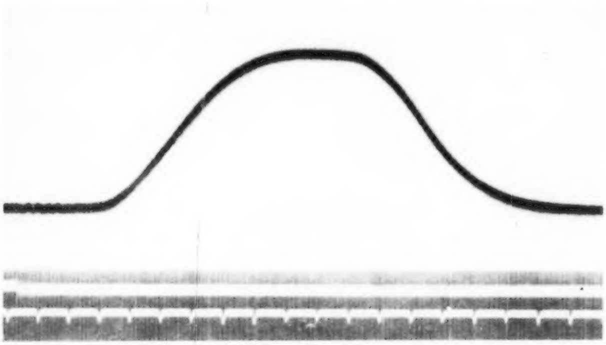

Fig. 1. The tension curve of a single contraction of the isolated ventricle of the terrapin heart. The relaxation phase was measured from the end of the plateau. The time line denotes intervals of $\frac{1}{5}$ second.

intense process of shortening is set up which allows the muscle to develop tension more rapidly.

We have also analyzed some of our records for the purpose of determining the velocity of the phase of increasing tension at different stages in the contraction event. By doing this we have been able to compare the curve of moment of the pressure series with that of the control. The essential part of the procedure was to take the time required for the tension curve to reach 20, 40, 60, and 80 per cent of the total height in each series. In 10 experiments tabulated in this manner we obtained a gradient for the successive steps that indicated a practically identical course in both series. From these time-scale studies it follows, therefore, that the pressure effect is not confined to a particular part of the phase of developing tension. Moreover, these results suggest that the mechanism by which pressure augments the development of tension is not through a slowing-up

of the time scale of the contractile process, such as occurs, for example, when a muscle is cooled to a moderately low temperature, and it gives an augmented response that is much prolonged in duration.

The foregoing results have been obtained, however, with heart preparations that were subjected to pressures of about 1000 pounds per square inch. With pressures of approximately 1500 pounds, as employed in the experiments shown in table 2, the degree of augmentation obtainable from the heart amounts to 68.2 per cent, or an increase of about 27 per cent over that exhibited with the lower compression, and the contraction phase shows an increase of 8.1 per cent in duration. This prolongation of the phase of contraction may denote, we believe, certain alterations in

TABLE 2

The influence of high pressure on the duration of the phases of contraction and relaxation of ventricular muscle

EXPERIMENT NUMBER	PRESSURE	STIMULATION (TENSION)	DURATION OF PHASES			
			Contraction		Relaxation	
			Control	Pressure	Control	Pressure
	pounds	*per cent*	*second*	*second*	*second*	*second*
112	1,600	100.7	1.16	1.30	1.33	1.47
113	1,600	83.2	0.97	1.15	0.88	1.12
114	1,600	55.0	0.83	0.84	0.92	1.17
116	1,600	45.1	1.42	1.44	1.53	1.62
117	1,600	40.9	1.46	1.59	1.26	1.44
119	1,500	50.0	1.25	1.40	1.35	1.46
122	1,500	100.0	1.35	1.44	1.23	1.46
123	1,500	42.7	1.42	1.48	1.78	1.85
125	1,500	114.3	0.89	1.05	0.89	1.19
126	1,500	50.0	1.54	1.58	1.58	2.06
Average..	1,550	68.2	1.23	1.33	1.28	1.48
			8.1%		15.6%	

the physical properties of the muscular system which begin to appear at the higher pressures and which serve to diminish or completely annul the increased velocity of tension development shown by the muscle at the lower pressure.

An additional feature of interest is contained in a series of 7 experiments in which we used the auricles of the turtle in place of the ventricles. These results are shown in table 3. The technic was the same excepting that both auricles were connected in parallel to the tension lever. These preparations compressed with 1500 pounds gave an average increase in tension of 142.5 per cent as the pressure effect, but the phase of contraction was prolonged by only 9.5 per cent over the average figure for the control.

The relaxation phase is increased with all grades of compression that we have used and, unlike the contraction phase, there appears to be a fairly direct relationship between the amount of prolongation of relaxation time and the degree of augmentation produced by pressure. It will be seen in tables 1, 2, and 3 that the average values for the increase in tension with pressure, expressed on the percentage basis, are as follows: 41.8, 68.2, 142.5, and that the corresponding figures for the percentage increase in the duration of relaxation are 9.4, 15.6, and 35.1. These data bear a proportional relationship to each other that is expressed approximately by the figure 4.2, but the evidence is not sufficient to warrant assigning this value as the probable mathematical constant. The facts noted are of

TABLE 3

The influence of high pressure on the duration of the phases of contraction and relaxation and on the rate of the rhythmically beating turtle auricle

EXPERIMENT NUMBER	PRESSURE	STIMULATION (TENSION)	DURATION OF PHASES			
			Contraction		Relaxation	
			Control	Pressure	Control	Pressure
	pounds	*per cent*	*second*	*second*	*second*	*second*
27	1,550	129.9	0.29	0.30	0.33	0.58
27b	1,550	148.7	0.31	0.33	0.47	0.60
28	1,525	195.5	0.30	0.36	0.38	0.64
29	1,525	112.0	0.40	0.43	0.76	0.89
30	1,500	132.4	0.31	0.38	0.46	0.61
31	1,400	133.3	0.38	0.39	0.76	0.88
32	1,425	145.5	0.29	0.31	0.35	0.54
Average..	1,496	142.5	0.326	0.357	0.501	0.677
			9.5%		35.1%	

interest in suggesting that the underlying phenomena of relaxation are modified in their time-course in a fairly regular way, but the part played by the different factors in the production of a prolonged relaxation phase cannot be put down with certainty at this time. It is quite clear that part of the lengthened relaxation time must result from the greater height at which the curve of tension starts in the pressure series, and it seems equally evident that if the pressure slows the contraction phase through an effect on the physical properties of the muscle system, then these same alterations will influence the relaxation phase in a similar manner. Experiments in progress, which employ much higher grades of compression, will throw additional light, we hope, upon the question of the viscosity changes in muscle when it is subjected to pressure.

SUMMARY

The isolated ventricle of the heart of the terrapin was arranged so that the tension of isometric contractions was optically recorded from a steel spring lever. With this method the heart muscle, subjected to a compression of about 1000 pounds per square inch, acting upon it through a completely surrounding liquid system, shows an augmented response when stimulated about 42 per cent over the tension developed by the same preparation at atmospheric pressure.

A compression force of about 1500 pounds, acting on the ventricle, gave an average augmentation of the tension response of about 68 per cent, and with preparations employing the auricles acting together the same degree of compression gave an increase of about 142 per cent in the power of developing tension.

The contraction phase of the ventricle exhibits very little increase in duration associated with a "pressure effect" of 42 per cent on the tension, but with higher grades of compression, that give an increase in tension of 68 per cent, the phase of contraction shows a prolongation of 8 per cent. It is suggested that the higher pressures may produce definite changes in the viscous-elastic properties of the muscle.

The phase of relaxation exhibits a definite prolongation by pressure. The average increase in duration amounts to 9.5 per cent at a pressure of about 1000 pounds, and 15.5 per cent at a pressure of about 1500 pounds per square inch.

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THE INFLUENCE OF HYDROSTATIC PRESSURE ON THE CONTRACTION OF CARDIAC MUSCLE IN RELATION TO TEMPERATURE[1]

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An adequate explanation for the important action of hydrostatic pressure on the contraction of cardiac and skeletal muscle must await the accumulation of more data concerning the conditions modifying its influence. While pressures of the order of 1000 pounds per square inch result in a striking augmentation of the tension developed by the muscle in response to a single stimulus, there is great variability among different preparations in the degree of stimulation. This is illustrated by the data given in previous papers (1928) in which a given pressure has resulted in an increase of tension as little as 20 per cent and as great as several hundred per cent. This variability is also abundantly illustrated in the control values for the experiments recorded in the present paper. However, any given preparation under constant conditions responds in a very uniform manner to repeated applications of pressure.

The variability of the degree of stimulation resulting from the action of pressure points to the desirability of studying in turn the various factors of the environment in relation to the pressure influence. Such a study has been started, and the present paper reports the results of the first environmental factor investigated, that of temperature.

More than twenty years ago Bernstein (1908) published an important paper in which he showed that the tension developed by the gastrocnemius muscle of the frog during a single twitch bears an inverse relationship to the temperature. His data indicate that this result holds in nearly all instances in which the strength of the stimulus is adequate to excite all the fibers of the muscle in the temperature range between 0°C. and 30°C., i.e., the response of the muscle has a negative temperature coefficient. On the other hand, the usual temperature coefficient typical of chemical reactions was observed for the tension plateau resulting from tetanic stimulation. The earlier but less exact investigations of Schmulewitsch

[1] An abstract of part of the work reported in this paper was printed in the Proceedings of the XIIIth International Physiological Congress, This Journal, 1929, xc, 337.

(1868), Gad and Heymans (1890), and Fröhlich (1907), which are critically discussed in Bernstein's paper, point to a similar conclusion. More recently Hartree and Hill (1921) have investigated the problem, both in relation to the tension developed and the initial heat production in the sartorius muscle of the frog. Their results essentially confirm those of the earlier workers and show that, as the temperature is lowered from 20°C. down to 0°C., there is a gradual increase in the tension developed in response to a single stimulus, and also that the heat production is correspondingly altered, with the result that the heat-tension ratio remains constant. In the case of the tension of the sustained contraction a Q_{10} of about 2.8 is reported, the heat production, however, is relatively small at the lower temperature, and the muscle thus maintains its force with greater efficiency.

Using an isotonic technic, which is poorly adapted for measuring muscular work, Clark (1920) observed an increase in the amplitude of the isolated frog heart as the temperature was decreased from 30° to 17°C. Similar experiments reported in the same year by Eckstein (1920) for temperatures between 7° and 32°C. also showed an inverse relation to the temperature. Doi (1920) has carried out experiments on the effect of temperature changes on the intraventricular pressure developed by the frog heart during contraction. In the range between 15° and 5°C., so long as the muscle was under physiological conditions with respect to the initial tension, the tension was considerably larger at the lower temperature. In conformity with the results of Doi it will be shown below that the tension developed by the ventricular muscle of the turtle is greatly increased as the temperature is lowered. We have carried out a series of experiments, which it is the purpose of this paper to report, in which the combined effects of low temperature and high pressure have been studied.

METHOD. The observations were all made on the isolated ventricle of the turtle, from the base of which a strip of muscle was cut for the purpose of abolishing spontaneous activity. Through the use of an optically recording isometric lever, photographs were obtained of the response of the muscle to single induced electric shocks at intervals of one minute. Details are given in our earlier papers (1928), together with a description of the chamber employed and the methods of applying pressure. The muscle was immersed in Ringer's solution, with which the entire chamber was filled, and through which pressures of from 1000 to 1600 pounds per square inch were transmitted. The temperature of the chamber was recorded by means of a galvanometer connected in series with a thermocouple, one junction of which was placed within a metallic tube extending into the chamber. The desired temperatures were obtained by placing the pressure chamber containing the muscle in an iced water bath.

In carrying out an experiment the following procedure was followed: As soon as the muscle was mounted a series of about three control contractions was recorded, following which the pressure was applied for a short period during which time another series of contractions was recorded. The preparation was then gradually cooled to the desired temperature and further photographs secured of the response before and during the application of pressure. Finally the temperature of the muscle was returned to that of the room and the influence of pressure again determined.

Results. The experimental results fall into three groups according to the degree of cold and pressure employed. In table 1 are tabulated the results of the first series of experiments in which moderate cold (10° to 16°C.)

TABLE 1

The combined influence of moderate pressure and moderate cold on the tension developed by ventricular muscle

See text for explanation

EXPERIMENT	PRESSURE	TEMPERATURE (COLD)	STIMULATION			
			Pressure, room temperature	Cold	Pressure, cold	Pressure, room temperature
	pounds	°C.	*per cent*	*per cent*	*per cent*	*per cent*
135	950	10.6	40.7	22.2	55.5	29.6
136	950	13.3	46.6	46.6	86.6	40.0
137	950	13.9	34.4	12.5	40.6	
138	950	13.8	33.3	29.1	52.1	14.6
139	950	13.4	25.6	18.6	39.6	14.0
141	950	13.6	18.5	8.0	18.5	18.5
35	950	10.0	43.1	34.5	63.8	44.8
39	950	16.0	38.9	30.5	69.4	
Average	950	13.1	35.1	25.3	53.3	

and a relatively low pressure (950 pounds) were employed. In this and subsequent tables the actual temperature and pressure for each experiment are given in the first columns. Under the heading *stimulation* there are four columns, the first of which gives the percentage increase of tension resulting from pressure alone (i.e., at room temperature), the second the increase due to cold alone, the third the increase resulting from the combined influence of pressure and cold, while the last column gives the pressure increase obtained after the preparation was returned to room temperature. In each of these four columns the data are expressed in terms of the percentage increase in tension in relation to the control value which was always obtained at room temperature and atmospheric pressure.

The following significant facts are brought out by the figures in the first table: Chilling the muscle from room temperature to a temperature averaging 13.1°C. resulted in an average increase in the tension developed during the response to a single stimulus of 25.3 per cent. At room temperature a pressure of 950 pounds per square inch resulted in an increase in tension of 35.1 per cent, whereas in the cold muscle this degree of pressure brought the maximum tension to a point 53.3 per cent above the control value. In other words, while the absolute increase in tension

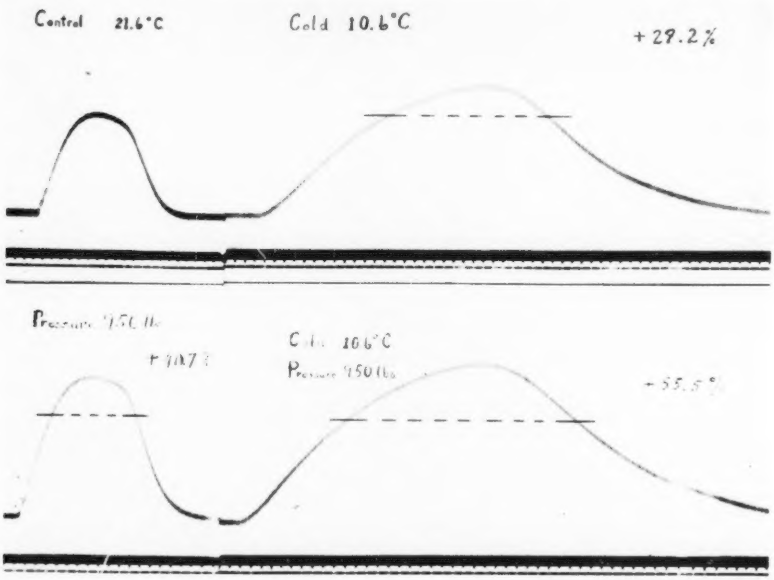

Fig. 1. The influence of pressure and temperature on the development of tension in the ventricle of the turtle. The lower record in each photograph gives the time in fifths of a second.

resulting from the pressure is less at the lower temperature, the pressure influence is largely superimposed upon the stimulation due to cold, so that the effect of cold and pressure acting together is far greater than either alone. Each individual experiment shows qualitatively the same relationship as that brought out by the average for the group. Both the cold and pressure effects are reversible, so that upon returning the preparation to room temperature the application of pressure again calls forth a stimulation which frequently corresponds closely to that obtained at the beginning of the experiment. This is shown in the last column of the table and in a more satisfactory manner in the last column of table 2.

A photograph typical of this group of experiments is reproduced in figure 1. In succession it shows the control response, its modification by cold, the effect of pressure, and finally the combined influence of cold and pressure. Broken horizontal lines have been drawn in each photograph at the level representing the control tension, and the figures at the left give the percentage increase above this value. While the two physical agents under discussion, cold and pressure, act similarly with regard to their influence on the energy set free during a simple contraction, their influence on the duration of the phases of contraction and relaxation shows no such similarity, as is clearly shown in the photograph. Pressures of this magnitude, as we have recently shown (Edwards and Cattell, 1930),

TABLE 2

The combined influence of high pressure and moderate cold on the tension developed by ventricular muscle

EXPERIMENT	PRESSURE	TEMPERATURE (COLD)	STIMULATION			
			Pressure, room temperature	Cold	Pressure, cold	Pressure, room temperature
	pounds	°C.	*per cent*	*per cent*	*per cent*	*per cent*
123	1,500	9.3	42.7	17.1	28.5	37.1
124	1,500	9.3	50.0	30.8	50.0	57.7
126	1,500	10.5	50.0	23.7	55.3	50.0
127	1,500	10.0	55.5	22.2	44.4	44.4
128	1,575	11.5	53.3	31.7	46.6	66.6
129	1,500	13.7	41.2	14.7	44.1	44.1
131	1,350	14.8	30.8	7.7	34.6	
132	1,325	10.1	61.1	44.4	61.1	61.1
133	1,300	14.0	48.4	38.7	61.3	45.2
134	1,300	14.5	39.7	10.3	47.1	35.3
Average	1,435	11.8	47.3	24.1	47.3	49.1

are almost without influence on the duration of the phase of contraction and but slightly increase the period of relaxation, thus contrasting with the effect of cold.

In the second group of experiments, summarized in table 2, relatively high pressures were employed, but the conditions otherwise were kept the same as in the first series. About the same increase in tension, 24.1 per cent, was brought about by moderate chilling, but, on the other hand, the higher pressures resulted in a correspondingly greater stimulation, averaging 47.3 per cent. However, the pressure applied to the cooled muscle brought the tension only to about the same level as was reached at room temperature, and since the level of tension developed had already been raised 25.1 per cent by the cold, the pressure effect was both relatively

and absolutely much less than at room temperature. It thus appears that the summated action of cold and pressure, observed in the first series of experiments, can no longer be obtained under higher pressure when the response from that cause alone is relatively great.

In the final series of experiments (see table 3) pressures in the range between 950 and 1575 pounds were used, as in the previous series, but the cooling was carried farther to an average temperature of 5°C. In most of these experiments the increase in tension developed in the course of a contraction was less than that existing for intermediate grades of cooling, so that the average value was only 16 per cent above the control. This figure remains in the average exactly the same for the tension developed under pressure at the low temperature, although the same preparations

TABLE 3

The effect of pressure on the tension developed by ventricular muscle at low temperatures

EXPERIMENT	PRESSURE	TEMPERATURE (COLD)	STIMULATION			
			Pressure, room temperature	Cold	Pressure, cold	Pressure, room temperature
	pounds	*°C.*	*per cent*	*per cent*	*per cent*	*per cent*
128	1,575	5.0	36.1	11.1	8.3	33.3
129	1,500	5.6	41.2	29.4	32.2	44.1
131	1,350	3.9	30.8	7.7	0.0	
133	1,300	5.4	48.4	25.8	19.3	45.2
134	1,300	5.6	39.7	7.3	16.2	35.3
136	950	5.6	46.6	43.3	53.3	40.0
138	950	5.4	33.3	8.3	12.5	14.6
139	950	4.2	25.6	16.3	12.8	14.0
141	950	4.4	18.5	−5.3	−10.5	18.5
Average..	1,203	5.0	35.6	16.0	16.0	30.6

showed the usual increase in tension when the pressure was applied at room temperature both before and after cooling. In other words, the usual action of pressure which augments the tension developed by the contracting muscle is entirely abolished when the temperature is reduced to the neighborhood of 5°C. This fact is of much interest in connection with the elucidation of the mechanism of the pressure effect, and we are planning further quantitative studies of this phenomenon.

Discussion. We have considered from time to time the action of pressure in relation to the mechanism of contraction, but in view of the very limited number of facts thus far established and the present uncertainty concerning the fundamental causes of the shortening process in muscle, any hypothesis attempting an explanation of the pressure influence can be little more than guesswork. The data here presented seem to indicate

that the agents, cold and pressure, produce their effects independently of each other and, in so far as their influence on the time relations of the contractile response is concerned, in a qualitatively different manner. The observation that with higher pressures the summated action with cold can no longer be obtained is susceptible to several explanations. In the first place it might be that the limits of the contractile mechanism were reached, so that the effects of the two agents acting on the same machine could be less readily shown. This probably is not the anwer, however, for in some experiments (unpublished) on cardiac muscle we have found it possible to increase the contractile tension several hundred per cent through the combined action of pressure and epinephrine.

Possibly a clue to an explanation of the results here reported lies in the parallelism between the effects of high pressure and low temperature on physical systems, both of which have a tendency to constrain the freedom of internal motion, as has been emphasized by Bridgman (1929). On such a hypothesis one might suppose that a certain limitation in the freedom of molecular motion in some part of the muscle structure is favorable to an increased response, hence the stimulating action of moderate cold and pressure when acting singly and together. There must be, however, a limit to the value of this influence, for a muscle, if sufficiently cooled, shows a falling off in tension, and in the present experiments it is shown that the application of pressure to a cold muscle causes no further stimulation. In the case of the higher pressures employed at moderate temperatures the failure of their combined action to give a greater response than was produced by pressure alone would be explained by the assumption that the pressure alone was sufficient to produce an optimum influence on the system. If such a simple relationship as that here suggested accounts for the phenomena observed, the application of pressure to a sufficiently cooled muscle should result in an actual falling off in the tension developed. As a matter of fact there are indications that this may be true in the experiments given in table 3. The critical value for the pressure employed seems to be about 5.4°C. In temperatures slightly above this value pressure always resulted in a small increase in tension, while in those below it caused a falling off in tension. This point must be verified by further experiments, using higher pressures and lower temperatures.

CONCLUSIONS

1. The tension developed during the contraction of the ventricular muscle of the turtle increases as the temperature is lowered.

2. Moderate hydrostatic pressure (950 pounds per square inch) still exerts a large fraction of its usual stimulating action on the tension developed by the muscle after cooling to between 16 and 10°C., i.e., the stimulating action of cold and pressure are in large part summated.

3. With higher pressures (1300 to 1500 pounds) the tension reached by the cold muscle is no greater than at room temperature.

4. When the muscle is cooled to approximately 5°C. pressure no longer causes an increase in the tension developed during contraction.

5. It is suggested that the similarity of the influence of high pressure and low temperature on the freedom of molecular motion may account for the observed results.

We wish to express our indebtedness to the Director of the Marine Biological Laboratory, Woods Hole, Mass., for placing at our disposal the facilities for carrying out this work.

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THE CORRELATIVE ACTIVITIES OF THE ALIMENTARY CANAL OF THE FOWL

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The present investigation was undertaken in the hope that further light might be thrown upon the motor activities of the alimentary canal in fowls. All of these observations were made on decerebrate fowls, three weeks or more after operation. The operative procedure used in decerebration was described by Ashcraft (1929).

P. J. Hanzlik and E. M. Butt (1928) found that spontaneous peristaltic contractions of the circular and longitudinal muscles of the esophagus and the crop occurred after decerebration in pigeons.

With reference to the motor activities of the proventriculus and the gizzard of the pigeon, Rogers (1916) found that both of these organs in the hungry bird were vigorously and continuously contracting. He says, "So far as the gastric mechanism is concerned loss of the hemispheres means only that the gastric activities are no longer related to distant influences." In his conclusions, Rogers says, "In normal and decerebrate pigeons the crop when nearly empty and during subsequent starvation exhibits hypermotility or hypertonicity, which is usually periodic but may at times run continuously for several hours. Three kinds of visceral influences normally stimulate the restlessness of the decerebrate bird: hunger associated with hypermotility of the gastric mechanism; thirst; and sometimes intestinal impulses."

Of the gastric mechanism, the gizzard undoubtedly has received more consideration than the other organs. Stubel (1911), Rossi (1904), Magnan (1911, 1912), Mangold (1906) and Kato (1914) have made extensive and thorough studies of the intrinsic and extrinsic nerve supply to the gizzard by various methods. Mangold found that with a balloon in different parts of the gizzard, various types of contractions were recorded.

Cannon and Washburn (1911) later confirmed by Carlson (1912) established in man that the stomach contractions give rise to the sensation of hunger by the stimulation of afferent nerve fibres in the muscle layers.

Observations on the esophagus and crop of the normal and decerebrate fowl (Leghorn). Direct observations or records of decerebrate birds were not made until three or four weeks following decerebration, thus giving time for the parts to heal.

The feathers were plucked from the neck and over the crop of unoperated birds to observe directly the activities of the esophagus and the empty crop in the normal bird. Peristaltic waves were seen passing down the esophagus and over the crop. In addition much more rapid rhythmic contractions were noted in the lower part of the crop, as were found by Rossi (1904) and later by Rogers (1916) in pigeons.

Direct observations made on normal birds were repeated with decerebrate birds in which the skin was slit over the crop. A better view of the rhythmic contractions was observed, involving only the lower part of the crop. No appreciable difference was noted in the rate and rhythm before and after incising the skin and fascia. The rate of the slower peristaltic wave was variable, an average of one every four minutes. Twelve to seventeen seconds were taken for a wave to pass over the entire crop. The incision through the skin was continued orally, exposing the lower end of the first part of the esophagus. The bird was then given two cubic centimeters of water, noting the peristaltic wave passing down the esophagus, spreading out over the crop, to continue over the second part of the esophagus. An average of seven seconds was consumed from the time the bird swallowed until the wave reached the crop. Not infrequently, the crop was so constricted that the lumen was almost entirely obliterated. This same activity was observed by Rogers (1916) working with pigeons. With a decerebrate hen that had been fasted 24 hours, feeding one kernel of corn at a time, the peristaltic wave originating in the esophagus and continuing over the crop quickly carried the first few kernels into the second part of the esophagus. Further feeding of grain caused a relaxation of the crop. Solid food caused the crop to relax sooner than a like quantity of water.

Muscular activity of crop, proventriculus, and gizzard. The balloon was employed in further study of the crop, the proventriculus, and the gizzard, to ascertain whether contractions of the crop were responsible for periodic restlessness of the bird, also to determine if any other visceral influences were responsible.

Very thin rubber balloons were fitted over one end of a flexible rubber catheter, size no. 10. A small glass tube was fitted in the end of the catheter to prevent the tube collapsing when the balloon was tied on. Three were prepared in like manner. The bird was restrained, lying on its left side. An incision was made through the skin, fascia, and crop, just large enough to insert the balloon. The free end of the catheter was placed in the mouth of the operator, while with the hand the balloon was introduced through the fistula into the crop, gently forcing it upward and backward, near the origin of the second part of the esophagus. Moderate inflation of the balloon caused it to be picked up by a peristaltic wave and carried through the proventriculus, finally resting in the gizzard. The

second ballon was introduced in like manner, except that as soon as the pull of the proventriculus was felt, the catheter was secured in order to prevent the balloon passing onward to the gizzard. The third balloon was inserted in the crop, all three catheters were then attached to water

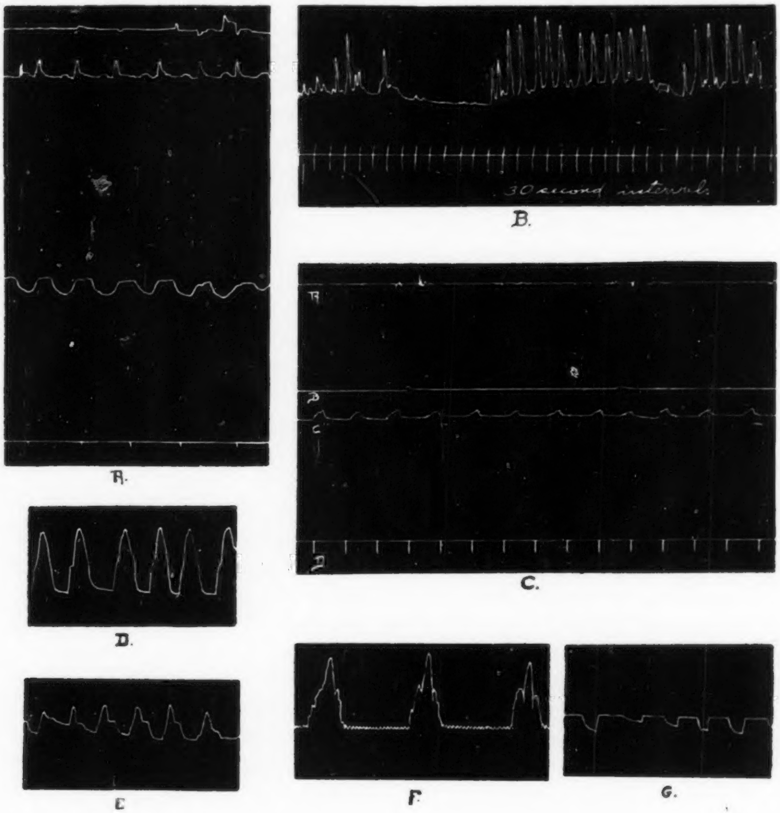

Fig. 1. Records of the decerebrate fowl, 24 hours' hunger.

A. Taking tracings in their order from above downward, crop, proventriculus and gizzard. Time: one minute intervals.

B. Crop: Balloon introduced through fistula in crop.

C. Upper tracing, movements of bird; middle tracing, crop contractions; lower tracing, gizzard contractions. Time intervals, one minute.

D. Gizzard: normal anacrotic curve.

E. Gizzard: dicrotic type of curve.

F. Gizzard: rare type of curve, dicrotic in addition to normal anacrotic curve.

G. Gizzard: plateau type of curve, rarely observed.

manometers from which simultaneous tracings were secured, recording the muscular activities of the crop, proventriculus and gizzard. After a record was secured, the bird was killed and embalmed, balloons in situ, and an autopsy performed to accurately determine the location of the balloons. The balloon of the proventriculus was the only one open to doubt, since the one in the crop was easily seen or felt. Considering the inlet and outlet of the gizzard, no trouble is encountered with a balloon in this cavity, in so far as its passage on to the duodenum is concerned. However, not infrequently the balloon may be punctured by gravel or other coarse ingesta, which of course necessitates a new start from the beginning in so far as the introduction of balloons is concerned. Records were secured by this trial and error method, one of which is reproduced in figure 1, A.

Records were obtained of the crop, proventriculus and gizzard, singly and in various combinations in the same bird, to determine if the presence of so much foreign material altered the activities of these organs.

Results. Considering first the crop movements, wide variations were found in the records obtained. Contractions occur singly or in groups of two to fifteen at intervals of one to forty minutes. Cannon (1911) observed in man, "During gastric digestion the stomach maintains its contractions with a considerable tonic tightening always existent." The tonus of the empty crop rises (fig. 1, B) during a series of contractions and falls following cessation of rhythmical activity.

Rogers (1916) and other investigators have described restlessness of pigeons as being associated with hunger. The same restless movement of hens was observed when a series of crop contractions occurred. To better correlate the activity of the crop and restlessness of the bird, a small, light wire cage was made to hold the decerebrate bird, this being attached to a pneumograph suspended from a supporting rod. The tambour connected with the pneumograph recorded the movements of the bird. Such simultaneous records (fig. 1, C,A) show that invariably hunger contractions and movement of the bird occurred at the same time. Sometimes movement of the bird would occur while the crop was quiescent; defecation would then take place, followed by cessation of restlessness.

All tracings of the glandular stomach show characteristic form, i.e., a rather slow, moderately high contraction, then more or less incomplete relaxation, followed by a further rise, more rapid and of greater intensity. This type of curve (fig. 1, A) corresponds very closely to the normal anacrotic curve of the gizzard as described by Mangold (1906). Rogers (1916) found that the proventriculus and the gizzard of the hungry pigeon were vigorously and continuously contracting. All records made of the activity of the proventriculus of the fasting fowl showed regular, constant contractions—an average of one per minute. Restlessness of the bird did not modify the activity of the proventriculus.

Numerous tracings were made of the activity of the muscular mechanism of the gizzard. Various types of curves were secured, depending upon the location of the balloon or balloons. The type of curve most frequently observed is termed by Mangold (1906) the normal anacrotic curve (fig. 1, D). Figure 1, E, shows a type of curve that is rather common, a tracing with a dicrotic wave, the anacrotic limb of the curve appearing smooth. Figure 1, F, reveals a rare type of curve, a dicrotic wave in addition to the normal anacrotic wave. Figure 1, G, shows still another type of curve, very rarely observed, which is called the plateau type by Mangold, who decided that in every position of the balloon, the first rise is due to the m. intermedii anterior and posterior, and the second to the m. laterales dorsalis and ventralis. The normal rhythm of the gizzard is 20 to 30 seconds. The gizzard of the fasting decerebrate bird was found to be vigorously and continuously contracting.

SUMMARY

1. Comparing by various methods, the activity of the crop of the normal fowl with that of the decerebrate bird, no appreciable difference was observed.

2. Further study of the decerebrate bird reveals that no material differences in crop movements were noted before and after incising the skin and fascia over the crop and esophagus.

3. A fistula through the skin and crop with balloons and catheters in situ did not appear to alter its activity.

4. Contractions of the empty crop invariably result in restlessness of the bird. Less frequently thirst and defecation are factors which also cause restlessness.

5. The form of the curve of contraction is always the same for the proventriculus.

6. Records obtained by the balloon method, show that the type of contraction of the gizzard is variable, depending upon the position of the balloon in the cavity of that organ.

7. In hunger, the proventriculus and gizzard are vigorously and continuously contracting.

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BLOOD CALCIUM AFTER SYMPATHECTOMY, ADRENIN INJECTIONS AND SHAM RAGE

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Calcium is an element of the utmost importance for various functions—for the growth of skeleton and teeth, for the maintenance of proper conditions of irritability of nerves and muscular tissue, for coagulation, and for other essential services. Ordinarily its content in the blood is fairly constant. Probably the constancy is under control, and there is some evidence that the nervous system exercises an influence upon it. Zondek (1922) has suggested that stimulation of the sympathetic leads to an increase of the calcium percentage. Billigheimer (1922) and Leicher (1922–23), on the other hand, report a slight fall of the Ca content of serum in clinical cases after giving adrenin subcutaneously. Recently Berg, Hess and Sherman (1928) have studied the effects of severance of sympathetic fibres and the vagi and have reported that there is a definite decrease in the calcium level of the blood after severance of the sympathetic fibres, amounting to nearly half the normal figure. They state, indeed, that it is only necessary to cut one of the splanchnic nerves in order to bring about this striking reduction. The lowest calcium level appeared usually from 1 to 24 hours after the operation and continued in some instances for a period of one or two weeks. In all their cases, however the normal level was ultimately regained.

The variations in the results reported by the observers mentioned above and the importance of nervous control of the calcium content of the blood, if there is such control, renders further observations desirable. Recent studies made in this laboratory have provided animals which have had the functioning of the sympathetic system excluded by removal of the system from the stellate to the pelvic ganglia on both sides. The proficiency obtained in operations on the sympathetic made readily feasible therefore, a special examination of effects of that system of nerves on the calcium content of the blood.

The method utilized for testing the calcium of the serum was one devised by Fiske and Logan (1930). The blood was taken by cardiac puncture.

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The calcium in 2 cc. of serum was precipitated by 1 cc. of 3 per cent ammonium oxalate. The precipitate was centrifuged, washed with 3 per cent ammonium oxalate by means of the centrifuge, and converted to calcium oxide. The calcium oxide was dissolved in 2 cc. of 0.02 N HCl and the excess acid back-titrated with 0.02 N NaOH by means of a micro-burette. Some duplicate determinations were made by means of the method of dry-ashing the serum. This longer method, however, yielded figures practically the same as those given by the shorter method and consequently the shorter method was usually employed.

By use of their method Trevan and Bainbridge (1926) found that the average calcium content of 100 cc. of blood of normal cats was 10.4 mgm., with variations ranging from 9.8 to 11.2 mgm. These results agree well with those recorded by Morgulis and Perley (1929) who found an average of 10.9 mgm., and also with those of Baumann and Kurlan (1927) who

TABLE 1

Calcium content of the blood serum of sympathectomized cats

CAT NUMBER	DATE	CALCIUM IN 100 CC. SERUM	REMARKS
	1929	*mgm.*	
417	June 16	10.20	Complete sympathectomy from stellate to pelvis, completed March 27, 1929
417	June 17	10.30	
422	June 18	10.80	
422	June 19	10.80	Same operation completed May 15, 1929
423	June 21	11.20	
423	June 22	11.40	Same operation completed May 15, 1929

found an average of 10.2, and with those of Stewart and Percival (1928) who found an average of approximately 10 mgm.

Observations were made on animals from which the sympathetic had been removed on both sides from the stellate to the pelvis. The results of these observations are shown in table 1. All but one of the figures lie within the range of variations of the normal. It is clear that absence of the sympathetic system does not modify to any noteworthy degree the calcium content of the blood.

Observations were also made on animals in which the right or left splanchnic nerves had been recently severed, as in the experiments by Berg, Hess and Sherman. As shown by the figures in table 2 this operation, performed on the *cat*, does not lower the calcium content of the blood—indeed, it does not alter it in any way to a noteworthy degree.

The effect of adrenin was tested by two methods. In four experiments it was injected intramuscularly. As shown in table 3, the effects of this

procedure, although varying and large doses were employed, were insignificant. Because of these negative results observations were made on

TABLE 2

Calcium content of the blood serum of cats before and after splanchnic section

CAT NUMBER	DATE	CALCIUM IN 100 CC. SERUM	REMARKS
	1929	*mgm.*	
12	July 16	10.80	Normal
12	July 16		Right splanchnics cut
12	July 17	11.20	26 hours after operation
12	July 18	11.00	48 hours after operation
12	July 20	11.30	4 days after operation
13	July 22	11.00	Normal
13	July 22		Left splanchnics cut
13	July 23	11.20	18 hours after operation
13	July 26	11.00	4 days after operation

TABLE 3

Calcium content of the blood serum after injection of adrenin

CAT NUMBER	DATE	CALCIUM IN 100 CC. SERUM		REMARKS
		Before	After	
	1929	*mgm.*	*mgm.*	
1	June 10	10.80	11.00	Intramuscular injection of 1 mgm. adrenin. Blood taken 1 hour thereafter. Respiration 38; pulse 145
2	June 11	10.60	10.30	Same injection as in cat 1. Blood taken 2 hours after injection. Respiration 40; pulse 150
3	June 27	10.20	10.30	Injection 0.5 mgm. adrenin every hour for 8 hours. Blood then taken. Respiration at end 42; pulse 152
4	June 29	10.40	10.20	Same procedure as in cat 3
5	June 20	10.80	10.80	Injected 0.001 mgm. adrenin per kilogram per minute for 27 hours
6	June 24	10.00	9.20	Same as cat 5, 24 hours
			8.00	After injecting 5 hours salt solution without adrenin
7	June 28	10.20	8.80	Two determinations after injection as in cat 5 for 31 hours
			8.70	
			10.00	17 hours later

animals in which adrenin was injected intravenously for long periods at the rate of 0.001 mgm. per k. per minute, by use of the method devised by Colwell (1930). Three animals thus injected, for 27, 24, and 31 hours

respectively (see table 3), showed in two instances a decrease in the calcium content. The animals were under amytal anesthesia. A test of the fluid input during the time, and the urine output, showed that there was a positive balance. In cat 6, for example, the input during 27 hours amounted to 339 cc. and the output was only 270 cc. For 5 hours thereafter salt solution alone was injected. This amounted to 75 cc. and the output during the period was only 3.4 cc. It was at the end of this period that the lowest calcium content of the blood was found. It seems probable, therefore, that the low calcium content in these experiments was not due specifically to the action of adrenin but rather to the large amount of fluid which was introduced with the adrenin and retained.

Other observations were made on animals which were exhibiting sham rage. As shown by the experiments of Cannon and Britton (1925) these animals have a very marked and prolonged activity of the sympathetic system with discharge of adrenin. In table 4 are presented the figures obtained under normal conditions before sham rage and after varying periods of exhibition of the pseudaffective phenomena. Again it is to be observed that there was no noteworthy change in the calcium content of the blood.

TABLE 4

Calcium content of the blood serum before and after an exhibition of sham rage

CAT NUMBER	DATE	CALCIUM IN 100 CC. SERUM		REMARKS
		Before	After	
	1929	*mgm.*	*mgm.*	
9	June 12	10.40	10.40	Sample taken 3½ hours after sham rage began. Respiration 140; pulse 250
	June 12		10.20	Two hours later
10	June 19	10.80	10.40	Sample taken 5 hours after sham rage began. Respiration 180; pulse 220
			10.60	One hour later

The negative results which are here reported raise questions as to how there might be such marked changes as have been reported by Berg, Hess and Sherman. The observations on prolonged injection of fluid containing adrenin, reported above, indicate the possibility that the phenomena observed by them may have been due to dilution of the blood. They argue against this explanation, to be sure, and as evidence they state that the hemoglobin percentages were only slightly changed by operation. They report observations, however, solely for their experiments on vagus section and not for those in which the sympathetic was sectioned. Unless the blood was actually proved not to be diluted, that possibility remains to

account for their results. The calcium is known to be present in the blood in two forms—as ionized calcium and in combination with protein. With a fall of blood pressure, such as might occur on severance of the splanchnic nerves, extracellular fluid comes in from the tissue spaces and thus adds volume and probably a corresponding amount of ionized calcium but not a corresponding amount of the calcium proteinate. In our experience dogs, which were used by Berg, Hess and Sherman, are more sensitive to splanchnic section and to manipulation of the abdominal viscera than are cats which were employed in the present experiments. Whatever the explanation, the results reported above indicate that in the cat the calcium of the blood remains fairly constant in spite of conditions which deprive the organism of its sympathetic nerve impulses in toto or in part (i.e., the splanchnics) or which cause a marked discharge of sympathetic impulses (as in sham rage) or which imitate the action of sympathetic impulses (by injecting adrenin).

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A COMPARISON OF THE TRIPLE EXTRAPOLATION (FICK PRINCIPLE) AND THE ACETYLENE (FOREIGN GAS PRINCIPLE) METHODS FOR THE DETERMINATION OF THE CARDIAC OUTPUT OF MAN

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The two chief methods in use for the determination of the cardiac output of man consist essentially 1, in determining the carbon dioxide or oxygen tensions of the arterial and mixed venous bloods and applying the Fick principle, or 2, in measuring the rate of absorption of a foreign gas. An examination of the results obtained by the numerous applications of these two principles reveals certain wide discrepancies. On the one hand, one finds results which indicate a value of about 2.2 liters as the cardiac output per square meter of body surface, per minute. Opposed to this lower value are certain data which indicate a much higher value as the cardiac output. It is true that many of the reported results may be dismissed on the grounds of obvious errors in the methods used. Other data are of no value for purposes of comparison because of the failure to maintain standard basal conditions. Thus determinations made without due regard to the disturbing effects of the ingestion of food, psychic disturbances, exercise, etc., will, as has been demonstrated (Grollman, 1929, b, c, d, e), give much higher values than those obtained under truly basal conditions. Nevertheless, there still remain data, not to be excluded by the above mentioned causes, which give totally irreconcilable results. The most dependable higher values are those reported by methods based on the Fick principle, particularly the large series of determinations of Field, Bock, and their collaborators (Field, Bock, Gildea and Lathrop, 1924; Field and Bock, 1925). In previous publications (Grollman, 1928, 1929 e) these higher values have been criticized. The lack of agreement between results obtained by the Fick and the foreign gas principle, nevertheless, would permit the justifiable suspicion that the fundamental assumptions of our methods for determining the cardiac output (by either the Fick or the foreign gas principle, or by both) might be erroneous. An actual experimental verification of the agreement of results obtained by both principles at the same sitting, would, therefore, add strong support to the contention that by certain present methods the actual cardiac output can be deter-

mined with a high degree of accuracy. The present communication records the success of this attempt.

Several previous observers have compared the Fick and the foreign gas principles (Boothby and Sandiford, 1916; Fridericia, 1918, Liljestrand and Lindhard, 1920; Eppinger, Papp and Schwartz, 1924). Unfortunately these are unsatisfactory since the methods used are open to objection, the data obtained are inadequate, and the comparisons were often made from determinations made on different subjects on different days. Thus, the conclusions of Boothby and Sandiford are based on experiments conducted at different times on different individuals; Fridericia's results were obtained on only two individuals; Liljestrand and Lindhard's comparison was made on two individuals at different times, and Eppinger, Papp, and Schwartz report the result of only a single comparison on one individual.

Methods employed. In the present work the results obtained by the use of acetylene have been compared with those obtained by the triple extrapolation method of Redfield, Bock and Meakins (1922). This latter method was adopted because aside from its sound theoretical basis (which is also shared by a number of other procedures based on the Fick principle) the method possesses the great advantage that it automatically causes the rejection of certain of the experimental results as being invalid. The method consists essentially in plotting three series of experimental points, the lines joining which should meet in a point. Failure to do so leads to rejection of the data.

The triple extrapolation method has been heretofore applied only twice. Barcroft and his collaborators (1923) made a few determinations by means of it during their expedition to the Andes. The results obtained were too few and apparently made with insufficient control of experimental conditions, to permit judging its value. Hayasaka (1927), using the same technique as Barcroft *et alii*, found on 7 normal subjects an average output of 2.2 liters per minute per square meter of body surface, which is identical to the value obtained on a large series of subjects using the acetylene method (Grollman, 1929e).

During the present use of the triple extrapolation method, certain difficulties arose which have not been heretofore described. The determination of only three experimental lines as suggested by previous workers, gave satisfactory results so infrequently that a great number of determinations had to be made before a successful determination was realized. Usually, as subsequent work showed, two of the curves were correct while the third (which was the decisive curve) was erroneous. In the present study six sets of determinations were made and of these, usually at least three (very occasionally four) of the lines met in a perfectly defined point. With this modification the mixtures used in the rebreathing were successively 4 liters of 1, nitrogen; 2, nitrogen + 50 cc. of oxygen; 3, nitro-

gen + 50 cc. of oxygen + 50 cc. of carbon dioxide; 4, nitrogen + 100 cc. of oxygen; 5, nitrogen + 100 cc. of oxygen + 100 cc. of carbon dioxide; 6, nitrogen + 200 cc. of oxygen.

Instead of the original apparatus of Redfield, Bock, and Meakins (1922) the arrangement shown in figure 1 was found to be convenient. In this figure, *A* is a five-way valve permitting the connection of the subject with the air through *B*; with the collapsible rubber tubes (about 2½ cm. wide), *C* and *E* or with the bag *D*. At the ends of *C* and *E* are attached Sadd valves, *I* and *J*. *C* is used for the collection of an alveolar sample after attaching a sampling tube at *F*. The rubber bag *D* is an ordinary short-necked anesthesia bag of 4 liters capacity into which is introduced the gas mixture whose composition has been described above. At the end of *E* is attached a rubber bag, *K*, of about 6 liters capacity. Sampling tubes are attached at *G* and *H*. The bag *K* permits the experimenter to note the

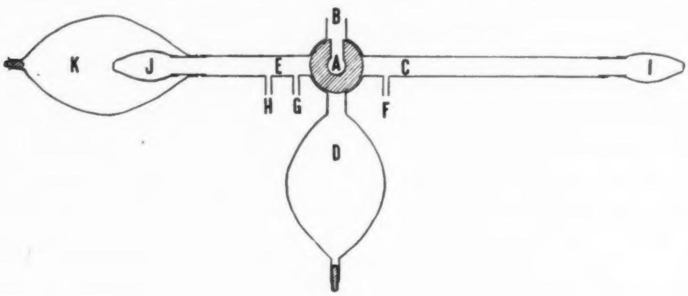

Fig. 1

amount of air expired by the subject. With untrained subjects failure to expire properly is noted and the analysis of worthless samples is thereby avoided. The subject either adjusts himself to breathing through the mouthpiece or better, immediately expires forcibly through *C*. The valve, *A*, is turned to communicate with the experimental mixture in *D*, and an inspiration, expiration, and a final inspiration are taken as described by Redfield, Bock, and Meakins (1922). The valve is then turned to *E* and the experiment concluded by the collection of the two samples at *G* and *H* in the manner described by the above named authors.

Figure 2 shows the results of a typical experiment. The lines in this figure represent the six sets of data obtained in successive experiments. It will be noted that the lines *1*, *2*, and *3* meet in a fairly well defined point. Were these three lines alone determined, one might be led to accept their point of intersection as representing the mixed venous blood. This point, however, is obviously absurd for the value of the venous carbon dioxide tension (37.5 mm.) is lower than the arterial tension which in the experi-

ment quoted was found to be 40.0 mm. The point of coincidence of the lines *1*, *4* and *5*, on the other hand, represents the true tensions of the mixed venous blood and was used in the calculation of the cardiac output. It should be noted that the oxygen tensions represented by the above two points do not differ markedly and either might have been used for calculating the cardiac output. This illustrates the danger of utilizing only the oxygen data for calculating the cardiac output by the triple extrapolation method. Line *6*, obviously, represents a worthless experiment.

Figure 2 demonstrates the difficulty of carrying out a theoretically simple rebreathing procedure. Despite the fact that all six determinations when plotted should have met in a single point, three determinations (2, 3 and 6) are obviously wrong and two of these (2 and 3) even agree. One may, therefore, justifiably suspect other rebreathing procedures of gross errors, particularly those methods in which duplicate results do not agree within the limits of the calculated technical errors. Fortunately in the triple extrapolation method we have a criterion for rejecting erroneous data. This is not true of other methods. A method of procedure which suggests itself in such cases is to carry out a great number of determinations and only use such as agree within the calculated technical errors.[1] Even then,

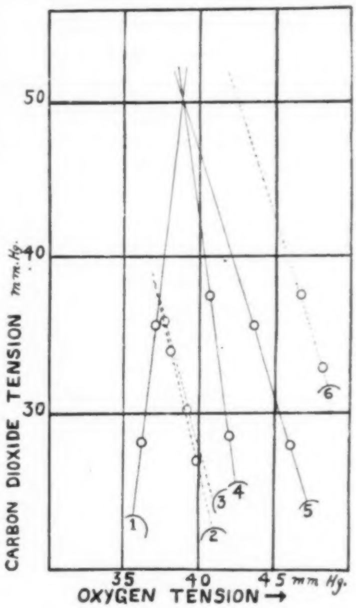

Fig. 2

[1] During the present investigation, several attempts were made to carry out the procedure of Field, Bock, Gildea, and Lathrop (1924) using the precautions suggested by Israëls and Lamb (1929). The results on two subjects, in whom single determinations of the carbon dioxide tension of the mixed venous blood agreed within less than a millimeter, agreed with that obtained by both the acetylene and triple extrapolation methods. In four other experiments, however, the individual determinations showed marked disagreement and as previously (Grollman, 1928) pointed out, the use of average values of several determinations in such cases is unwarranted. It should be emphasized, however, that the agreement of successive determinations is no absolute proof of the validity of a series of results. Certain individuals are capable of repeating a given procedure in such a way as to lead to constant but grossly erroneous results. This last state of affairs is rendered improbable in the triple extrapolation method where, due to the different mixtures used in the successive experiments, the conditions of the individual determinations are different.

however, one cannot be absolutely sure of the correctness of the result as is demonstrated in the case of lines *1*, *2* and *3* of figure 2. The fortuitous meeting of three lines in an obviously erroneous point, as occurred in the case cited in figure 2, was only encountered on one other occasion during the present investigation when its invalidity was easily manifest, as in the case of figure 2, by the absurd value of the carbon dioxide tension of the mixed venous blood.

To calculate the cardiac output one may utilize either the carbon dioxide or the oxygen data, using the corresponding dissociation curve for blood. For accurate work this curve would have to be determined for each subject investigated. However, for the purposes of the present paper a single curve has been considered adequate and that of Bock, Field, and Adair (1924) has been used for this purpose. Except for subject E. K. M., whose oxygen dissociation curve was known, the oxygen capacity of the blood was assumed to be 20 volumes per cent in the case of the male subjects, 18, in the case of the females, and the saturation of the arterial blood was assumed to be 96 per cent. All the subjects were normal, young, healthy individuals, and the assumption of these values will, therefore, probably introduce no large error.[2]

The determinations by the acetylene and the triple extrapolation methods on any one subject in a given posture were always made at the same sitting, during the course of about one hour. They were performed in the morning soon after the subject's arrival at the laboratory. The precautions, previously cited, (Grollman, 1929e) for attaining truly basal conditions were observed.

Results. The results of the present study are summarized in table 1. Considering the technical errors involved in the various methods used, and the assumptions involved in the use of a single dissociation curve, the results are all that can be desired. They indicate clearly that the Fick principle and the foreign gas principle yield results which are in perfect harmony (within the experimental error). Moreover, the results indicate the accuracy of the lower values for the cardiac output, as opposed to the

[2] Calculation of the cardiac output on the assumption of the probable variation in the oxygen capacity and arterial saturation of the blood of normal individuals gave results whose general agreement with the results by the acetylene method was about the same as is shown in table 1. We have also calculated the cardiac output using the curves and values for oxygen capacity given by Christiansen, Douglas, and Haldane (1914). The results using the latter data are, in general, not in as good agreement with those obtained by the acetylene method as when the curves and data of Bock, Field, and Adair are used, although, in some cases, the reverse is true. For the purposes of the present investigation, agreement, within 10 per cent, of the results obtained by the acetylene and triple extrapolation methods is satisfactory and hence we have considered it unnecessary to determine the individual dissociation curves for all the subjects studied.

higher values recorded by certain authors. The results also present further evidence for the relation of the cardiac output of normal young individuals

TABLE 1

A comparison of the results of determinations of the cardiac output by the methods of triple extrapolation (Fick principle) and the use of acetylene (foreign gas principle)

SUBJECT	SEX	HEIGHT	WEIGHT	SURFACE AREA	POSTURE	PULSE RATE	OXYGEN CONSUMPTION	CARDIAC OUTPUT: By triple extrapolation method		
								From CO_2 data	From oxygen data	By acetylene method
		cms.	*kgs.*	*sq. meters*		*per minute*	*cc. per minute*	*liters per minute*	*liters per minute*	*liters per minute*
A. G.	♂	164	71	1.77	Recumbent	60	230	3.54	4.07	3.84
					Sitting	60	240	3.80	3.90	3.83
					Standing	88	327	3.90	4.42	3.64
L. C. G.	♀	158	57	1.57	Recumbent	52	192	3.23	3.01	2.90
					Sitting	59	200	3.16	3.08	2.86
					Standing	65	230	2.94	2.80	3.01
L. M.	♂	164	57	1.62	Recumbent	74	190	3.37	3.40	3.20
					Sitting	78	194	3.57	3.46	3.34
					Standing	100	245	3.29	3.60	3.02
A. A.	♂	164	70	1.76	Recumbent	60	217	4.70	4.21	3.94
					Sitting	62	240	4.05	3.94	3.72
					Standing	75	252	4.30	4.06	3.60*
A. I.	♂	166	70	1.78	Sitting	68	251	4.23	3.80	4.00
C. C.	♀	156	56	1.55	Sitting	56	199	2.70	2.86	3.01
M. R.	♂	168	49	1.54	Sitting	62	189	3.56	3.70	3.10
A. D.	♀	149	52	1.45	Sitting	72	190	2.87	3.40	3.22
E. K. M.	♂	181	68	1.87	Sitting	67	239	4.22	3.82	3.62
G. F.	♂	180	70	1.89	Sitting	72	282	4.38	5.13	4.90
Average				1.68				3.66	3.70	3.48
Average per square meter of body surface								2.18	2.20	2.07†

* This result is probably too low due to an experimental error.

† The average value of the cardiac output per minute per square meter of body surface, as obtained by the acetylene method for the 10 subjects of the present investigation, is 2.12, for the sitting posture. This is in agreement with the value 2.21, previously obtained (Grollman, 1929 e) on 51 individuals in the sitting position, considering the difference in the number of subjects included in the two studies.

in the truly basal condition to the surface area as previously demonstrated by the acetylene method alone (Grollman, 1929e).

The results obtained on the first four subjects cited in table 1 clearly indicate the independence (within 10 per cent, which is considered as the

gross error in the limited experiments cited) of the cardiac output to postural changes. The supposed effect of posture on the circulatory rate has already been considered (Grollman, 1928) and the present results verify the previous conclusions. Field and Bock (1925) utilizing a method based on the Fick principle were the chief supporters of the view that posture markedly affects the cardiac output. The present results in which both the acetylene and the triple extrapolation methods substantiate the previous findings (in which nitrous oxide was used as the foreign gas), place in further disrepute those who maintain that postural changes are accompanied by a great change in the cardiac output.

The results of the present study lend additional support to the trustworthiness of the triple extrapolation method for determining the cardiac output. Unfortunately, the method entails much laborious analytical work (particularly when, as shown above, six sets of experimental determinations are made). It also requires, for exact work, the determination of the oxygen capacity and the dissociation curve of the subject's blood as well as the determination of the alveolar gas tensions. In untrained subjects, an accurate determination of the last function may be very difficult. However, the method may prove of value in certain cases where the simpler acetylene method is inapplicable. In such cases it would seem to be a much safer and more accurate, although more laborious method than the many simpler modifications of the Fick principle which have been suggested and used for determining the cardiac output of man.

The results of the present investigation by the acetylene method, and the data previously reported (Grollman, 1929e) (excluding subjects 11 and 38) give a total of 59 values for the basal cardiac output of normal young individuals in the sitting posture. The average cardiac output for this group is 2.18 liters per minute per square meter of body surface. The calculated probable error of a single observation is 0.11 liter and the probable error of the arithmetical mean of the whole series of 59 observations is 0.014 liter per minute per square meter of body surface.

SUMMARY

A series of determinations of the cardiac output of 10 normal, young individuals was made using the triple extrapolation (Fick principle) and the acetylene (foreign gas principle) methods. The results by the two methods agreed within the limits of the experimental errors. The previously reported independence of the cardiac output to postural changes was also verified by both methods. The triple extrapolation method was found to give reliable results if six sets of determinations were made (instead of three as suggested by its original proponents) and care was taken to avoid certain pitfalls encountered in its use.

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THE EFFECT OF ANAEROBIOSIS AND OTHER FACTORS ON THE OXYGEN CONSUMPTION OF IRRITABLE AND NON-IRRITABLE MUSCLES

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It has been stated by Meyerhof (1920; 1924, p. 52) that when muscles are brought into an atmosphere of oxygen after a period in nitrogen they consume an extra amount of oxygen at an increased rate until they have approximately made up for the oxygen which they failed to get during the nitrogen period. In nerves and frog skin this is not the case, the excess oxygen or the anaerobic oxygen debt being only 5 to 20 per cent of the oxygen which was missed during anaerobiosis. (Fenn, 1929.) A muscle must evidently possess a peculiarly effective mechanism for maintaining its supply of energy under anaerobic conditions. It seemed of importance therefore to confirm this statement of Meyerhof's which appeared to rest upon only three somewhat indirect determinations.

METHOD. For the oxygen measurements a differential volumeter was used. In some cases the carbon dioxide output was also followed by measurements of the conductivity of barium hydroxide in which the carbon dioxide was absorbed. (Fenn, 1928b.) Nitrogen was purified over hot copper and no rubber connections were used. Hydrogen, when used, was purified over hot platinized asbestos. To free the muscle chamber completely of oxygen these gases were passed through it for 20 to 30 minutes either steadily or in some cases intermittently. In some cases oxygen or air was admitted to the respirometer bottle containing the muscle after the anaerobic period with special precautions not to interfere with the oxygen readings for more than 10 to 15 seconds. (Fenn, 1929.) In other cases pure oxygen was introduced without such precautions and readings were resumed 10 minutes later after equilibrium had been reëstablished. Loss of time of this amount makes little difference in the final result because the excess oxygen consumption in the case of muscle lasts for many hours. Muscles were ordinarily suspended in the respirometer or merely resting on the bottom of the bottle in an atmosphere of oxygen but without being immersed in Ringer's solution. The experimental temperature was 22 to 23° C. The Ringer's solution used contained 0.65

per cent NaCl, 0.01 per cent KCl, 0.02 per cent $CaCl_2$ and sodium phosphate buffers, pH 7.3, 0.0008M.

Anaerobic oxygen debt. The first of these experiments were done in May 1928 and some of them are illustrated in figure 1. The two upper graphs,

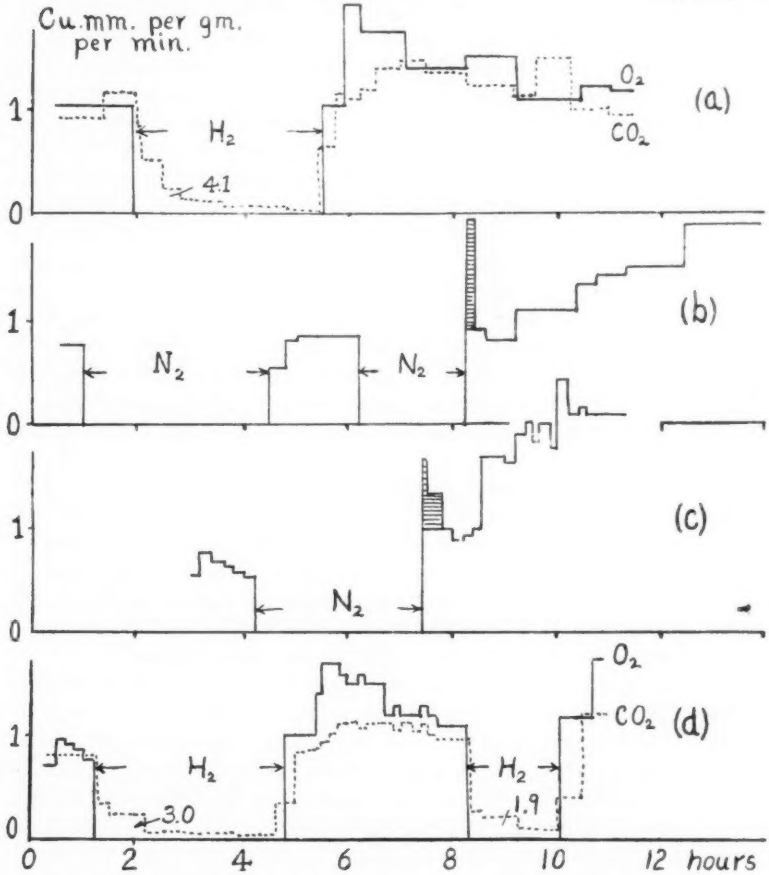

Fig. 1. Rate of oxygen consumption and carbon dioxide output (dotted lines) as ordinates against time in hours as abscissae. Experiments on 4 semitendinosus muscles. Muscles (a) and (b) are from one and (c) and (d) from another frog. Experiment of July 1928. Weights of muscles were 114, 199, 104, and 119 mgm. respectively. One half of (a) was removed to diminish its size. Shaded areas indicate correction calculated for absorption of oxygen by sodium hydroxide. The amount of anaerobic carbon dioxide is given in vols. per cent. (a) and (d) were at 21.5°C. while (b) and (c) were at 22.9°C.

a and *b*, are from the two semitendinosus muscles from the same frog. In the former case, CO_2 was also measured as shown by the dotted line. As would be expected, there was an anaerobic output of preformed carbon dioxide of 4.1 vols. per cent and in recovery the oxygen was in excess of the CO_2 by about the same amount, indicating presumably that a corresponding amount of lactic acid had been removed oxidatively. It is significant that the recovery carbon dioxide passes through a maximum at about 2 hours after oxygen was admitted. The initial R. Q. was 1.03 and after recovery it was somewhat less than 1.0. Recovery was presumably not quite complete even after 6 hours of recovery since the oxygen had not quite returned to its original level. The oxygen missed during hydrogen was 23.5 vols. per cent while the excess oxygen consumed in recovery above that expected at the initial rate of oxygen consumption (1.05 cu. mm. per gm. per min.) was 49.3 − 36.7 or 12.6 vols. per cent. This extra oxygen consumption has been called the anaerobic oxygen debt of the tissue. The recovery CO_2 output was 42 vols. per cent. Allowing for the anaerobic CO_2 this makes the recovery R. Q. 49.3/ (42 + 4.1) = 0.94.

In short, the behavior of this muscle (fig. 1a) is just what would be expected according to the Hill-Meyerhof theory. The match muscle in nitrogen, however, after a somewhat longer anaerobic period (interrupted by a short oxygen period) showed quite a different result. Immediately after oxygen was readmitted the oxygen consumption began to mount quite rapidly and continued to increase throughout the period of observation. Ten hours later it had not diminished again. A similar irreversible rise in oxygen consumption is shown in figure 1c. The experiment of figure 1d (match muscle to 1c) is very similar to that of figure 1a; the oxygen missed was 17.2 vols. per cent of which 10.6 vols. per cent was regained during recovery before hydrogen was again applied.

The most interesting feature of these experiments was the large increase in the oxygen consumption illustrated in figures 1b and 1c. This is not due to bacterial contamination which does not become appreciable for another 12 or 24 hours. This can be shown by control muscles and by making smears for microscopic observations. Interest in this result was heightened by the appearance soon after of a paper by A. V. Hill (1928) describing an increase in the resting rate of heat production of muscles kept for long periods in nitrogen. He was evidently observing the same phenomenon.

That a similar increase in the resting rate of oxygen consumption can occur without treatment with nitrogen was also shown by Mr. D. S. Martin in my laboratory (cf. Fenn, 1928) who observed simultaneous increases in the resting rate of heat production and the resting oxygen consumption and carbon dioxide output beginning 10 hours after dissection. Since a calibration of the muscle showed that the increase in the heat was completely explained by the oxygen consumption it was quite certain in this

case that there was no confusion due to condensation of water vapor such as Hill found in some of his experiments. Simple calculation shows that the observed oxygen consumption could not possibly be explained in significant amount by a change in the vapor pressure of the muscle. Such a change could only account for a fraction of a millimeter movement of the index drop.

In returning to this problem more recently, attempts were made to follow the contractility of the muscle together with its oxygen consumption changes in order to see whether this increase in oxygen consumption coincided with a loss of contractility. In this work the phenomenon of the non-irritable muscle was soon encountered and was confirmed in every way (Duliere and Horton, 1929). Muscles not previously soaked in Ringer's

TABLE 1

Oxygen consumption of muscle

Cubic millimeters per gram per minute

	WASHED	UNWASHED
	1.0	2.3
	0.5	0.9
	0.8	1.5
	1.0	3.0
	0.4	1.2
	0.8	1.0
	1.4	2.0
	1.2	1.8
	2.7	2.9
	1.4	1.1
	0.8	1.2
	1.2	2.2
	0.7	1.3
Average	1.07	1.72

solution would soon fail to contract but would recover completely after a few minutes in Ringer's solution. It was soon found that *this condition of non-irritability was associated with a high oxygen consumption* as shown in table 1. The figures there collected show that in 12 out of 13 experiments the unwashed non-irritable muscle consumed oxygen faster than the washed muscle, the average increase being 1.7 times. Duliere and Horton (1929) were unable to discover any chemical difference between irritable and non-irritable muscles but there is most certainly a difference in oxygen consumption which is therefore not associated with a corresponding change in lactic acid or phosphate metabolism. It might be interpreted as an increase of permeability of the muscle cells demanding consequently a greater consumption of oxygen to maintain the viability of the

cell. The sub-normal electrical conductivity of non-irritable muscle found by Thomson (1928) does not however suggest an increase in permeability.

In later attempts to measure the anaerobic oxygen debt special attention was paid to the previous soaking of the muscle in Ringer's solution. The results of three such experiments with washed muscles are shown in figure 2. The rate of oxygen consumption is plotted against time. To test the irritability the muscles were removed from the respirometer at intervals and were stimulated with an induction coil in the usual way. Black circles indicate normal irritability. Unblackened circles indicate non-irritability. By partially blackened circles an attempt is made to illustrate the degree of irritability observed. In the first two experiments 4 and 3 hours in hydrogen respectively failed to alter the irritability of the

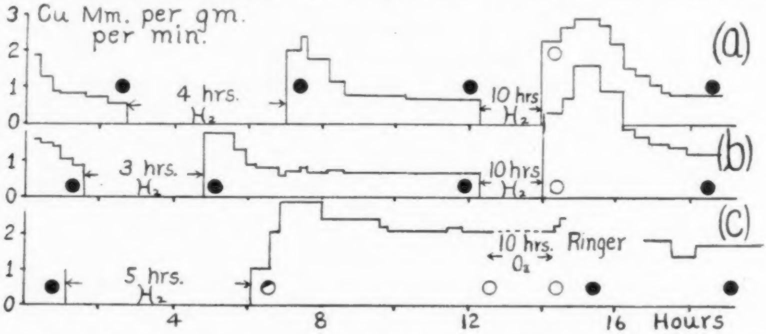

Fig. 2. Rate of oxygen consumption of three sartorius muscles plotted against time. Black circles indicate normal irritability; empty circles indicate no response. The muscles are those listed as nos. 3, 2, and 8 respectively in table 2. Temp. 22.8°C. Weights 166, 167, and 131 mgm. respectively. Muscles (a) and (b) were match muscles from the same frog. August, 1929.

muscles (2 sartorius muscles from the same frog). The magnitudes of the oxygen debts recorded were somewhat less than the amounts of oxygen missed, 73 and 63 per cent respectively. Thereafter both muscles were put into hydrogen again for 10 hours after which both were found to be non-irritable and consuming oxygen at a very high rate. After 4 hours' recovery in oxygen however both muscles contracted again and the rate of oxygen intake had decreased nearly to normal resting values. The excess oxygen used in these cases was 98 per cent and 70 per cent respectively of the oxygen missed. Long anaerobic periods are obviously necessary for the asphyxia of a resting muscle in which the energy demands are relatively so small. These two experiments are therefore quite in accord with theoretical expectations and offer nothing new.

Figure 2c however represents an experiment in which some other factor

is involved for the muscle becomes non-irritable after only 5 hours in hydrogen and does not recover irritability after 17 hours in oxygen during which time the amount of extra oxygen consumed was probably 10 times as great as the oxygen actually missed. After a few minutes in Ringer's solution however the irritability returned promptly to normal and the oxygen consumption fell slightly from 2.0 to 1.6 cu. mm. per gram per

TABLE 2

Anaerobic oxygen debt of muscle

NUMBER OF MUSCLE	ANAEROBIC PERIOD	OXYGEN PERIOD	O_2	O_2 MISSED	O_2 REGAINED	PER CENT REGAINED
	hours	*hours*	*cu. mm. per gram per minute*		*vol. per cent*	
1	2.0	3.0	0.74	8.8	5.4	61
2 (a)	3.0	7.0	0.7	12.4	7.8	63
2 (b)	10.0	4.8	0.7	42.0	41.4+	98+
3 (a)	4.1	5.0	0.7	17.2	12.5	73
3 (b)	10.0	4.8	0.7	42.0	29.5+	70+
4	2.1	3.2	0.74	8.9	13.7	154
5	3.5	4.5	0.9	19.0	33.4	176
6	2.1	5.5	0.74	8.8	15.7	178
7	2.3	9.0	0.6	8.3	15.8	190
8	5.0	7.5	2.1	18.0	60.0+	330+
9	2.7	5.6	0.62	9.9	58.8	594
10	9.0	8.0	(14°C.)	15.4	11.2	73 *
11	8.5	10.5	(14°C.)	14.7	12.9	88 *
12	2.5	3.5	(22°C.)	9.8	13.5	138 *

Brackets indicate match muscles from the same frog.

Nos. 2b, 3b and 5 regain irritability after recovery period; no. 8 only after soaking in Ringer's solution. All others contract, throughout the experiment.

Nos. 2, 3 and 8 are plotted in figures 2, b, a, and c respectively.

Nos. 10 and 11 were at 14°C.; all others at 22° C.

All these muscles were initially soaked at least 15 minutes in Ringer's solution.

The gastrocnemius was used in nos. 10, 11 and 12, the semitendinosus in no. 9 and the sartorius in all others. The plus sign (+) indicates that the rate of O_2 consumption was still above the basal rate at the end of the experiment.

* Meyerhof (1920).

minute. Possibly in this muscle the preliminary soaking had been inadequate and it behaved like a non-irritable unwashed muscle.

In table 2 are collected together a number of experiments in which the oxygen missed during a previous period of anaerobiosis is compared with the oxygen regained during a succeeding period in oxygen. The duration of these periods in nitrogen and oxygen is also given and the basal rate of oxygen consumption in cubic millimeter per gram muscle per minute. The three experiments of Meyerhof (1920) have been recalculated and are

included at the end of table 2. In his experiments the duration of the anaerobic period and hence the oxygen missed was calculated from the lactic acid concentration of the muscle at the end of the anaerobic period and from the average rate of accumulation of lactic acid known from other experiments. The result is therefore somewhat indirect. The experiments of table 2 are arranged in order according to the percentage of the oxygen missed which is regained during recovery. The per cent regained varies all the way from 61 per cent to 594 per cent. In frog nerve and frog skin the per cent regained varies only from 5 to 25 per cent (Fenn, 1929). Muscle evidently possesses a unique mechanism for surviving in nitrogen.

How shall one interpret this large variation in the percentage of the oxygen missed which is regained? It would seem likely that there might

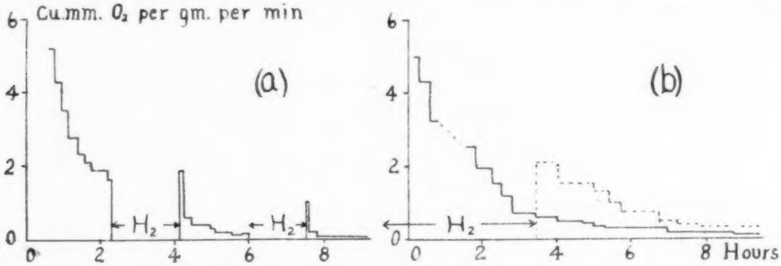

Fig. 3. The effect of hydrogen on the oxygen consumption rate of muscle mash. (a) 394 mgm. muscle finely cut up in 1 cc. Ringer's solution. (b) Two samples of 289 mgm. and 298 mgm. (dotted line) finely cut up in 0.5 cc. Ringer's. The latter sample was put directly into hydrogen after preparation. Temp. 22.9°C in (a) and 22.6°C. in (b).

be some correlation with the glycogen content of the muscle. Muscles poor in glycogen tend to go into rigor early (Hoet and Marks, 1926). Anaerobiosis precipitates rigor. This process possibly involves other reactions than a lactic acid formation and may lead to a high oxygen consumption.[1] Muscles rich in glycogen or otherwise in good condition may then go into nitrogen without involving any other reaction than the formation of lactic acid. Normally at the beginning of the anaerobic period the rate of lactic acid formation is the same as its rate of formation during oxygen; it accumulates merely because it is not oxidatively removed (Meyerhof). If this remained true throughout the anaerobic period and if the oxidative quotient remained unchanged after anaerobiosis, then the

[1] Abderhalden and Wertheimer (Pflüger's Arch., 1923, cc, 176) have shown a marked increase in the reducing power of muscles in rigor which correlates well with a high oxygen consumption.

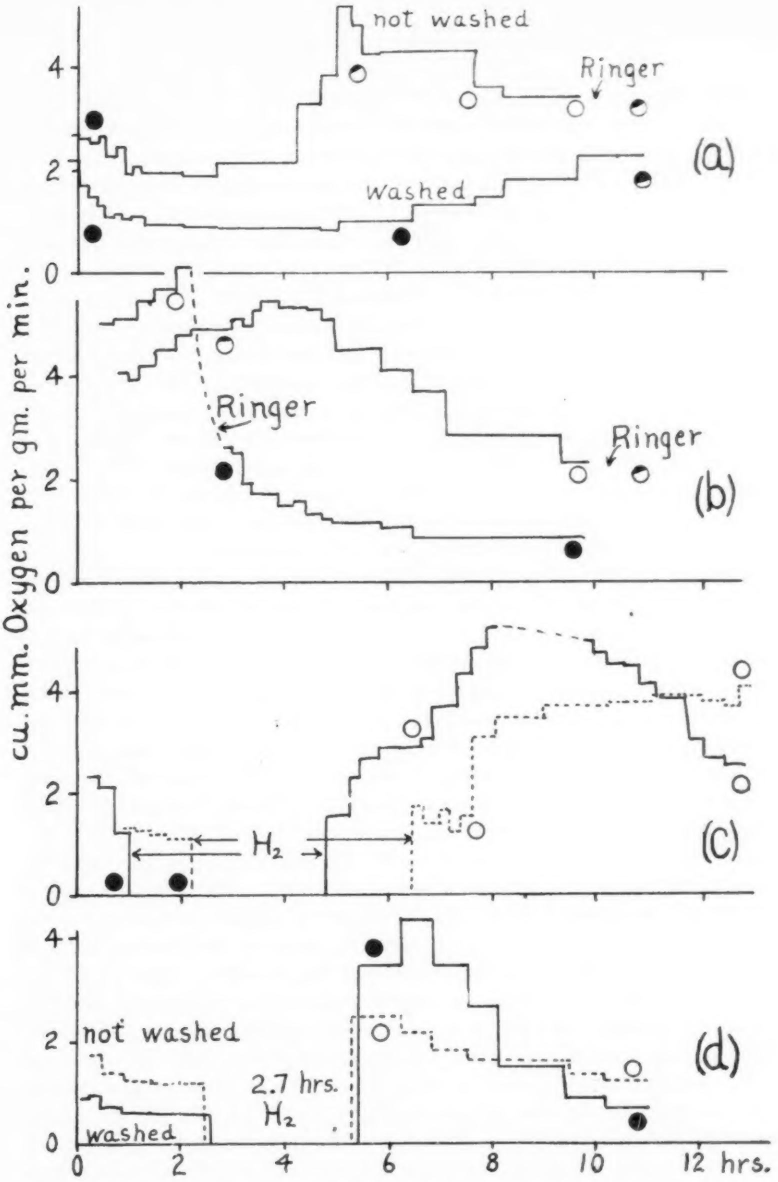

Fig. 4. Rates of oxygen consumption of washed and unwashed muscles. (a) semitendinosus muscles 110 and 103 mgm. (washed). 24°C. 31/8/29. (b) semitendinosus muscles 80 and 81 mgm. 24°C. 28/8/29. (c) sartorius muscles, 135 and 137 mgm. (dotted), the former suspended in oxygen and the latter in 0.3 cc. of Ringer's solution. 22.8°C. 14/7/29. (d) sartorius muscles, 139 and 157 mgm. (washed). 24°C. 27/7/29.

recovery oxygen should equal the oxygen missed. If the rate of lactic acid formation is somewhat diminished toward the end of the anaerobic period then the oxygen regained even after complete recovery might be somewhat less, as it normally is, than the oxygen supposed to have been missed. This would involve then a slight decrease in the rate of glycolytic energy turnover in the muscle during nitrogen, as is so markedly the case in nerve, and would explain the fact that in some muscles only 60 per cent of the oxygen missed is regained. On the other hand muscles in poor condition or depleted of glycogen may be forced to draw upon more expensive sources of energy for survival and may suffer in consequence more or less irreversible changes in nitrogen. This would explain Hill's high survival heat rate in nitrogen and a high oxygen consumption in recovery. What the nature of these other reactions may be is not now known. The case of no. 9 in table 2 (cf. also fig. 4d) is interesting in this connection. The oxygen regained was 6 times that which was missed and yet the irritability of the muscle was never markedly diminished and recovery was complete as far as the rate of oxygen consumption was concerned.

Meyerhof (1920) makes the statement that exposure of muscle to nitrogen may diminish the oxygen consumption after recovery to less than the normal rate and cites this as evidence of the injurious effect of lack of oxygen on the tissue. I have never observed this in any of my experiments except as the gradual fall in the basal rate with time might be interpreted in this sense. I have never seen the oxygen consumption after nitrogen lower than in a control muscle kept in oxygen for a similar length of time. The general rule seems to hold that the lower the oxygen consumption the better the condition of the muscle. In nerve however I have observed a very slight effect of the sort which Meyerhof describes (Fenn, 1930).

Anaerobic oxygen debt in muscle mash. Several attempts were made to measure an anaerobic oxygen debt in a muscle mash. The result of a typical experiment is shown in figure 3a. The rate of oxygen consumption decreases rapidly but is very high at the start, being about 6 times the basal rate of oxygen consumption usual in intact frog muscles. After a 2 hour period in nitrogen the small temporary increase in the oxygen consumption is not large enough to be of significance and the rate then falls to a still lower level. A subsequent hydrogen period has the same result. No increase in the oxygen consumption after anaerobiosis can be demonstrated in this way but figure 3b shows an experiment with two similar samples of muscle mash one of which was left in oxygen while the other was put into hydrogen for over 4 hours. When oxygen was again admitted after this more prolonged anaerobic period the rate of oxygen consumption was definitely larger than in the control mash. A repetition of this experiment gave an identical result. The sample kept in hydrogen does not however regain anywhere near as much oxygen as it missed during the anaerobic period.

The decrease in the rate of oxygen consumption of the muscle mash in oxygen is explained, according to Meyerhof, by the loss of materials from the muscle fragments by diffusion. He endeavored to prove this by the fact that the decrease in oxygen consumption was prevented or much delayed if the tissue was suspended in boiled muscle extract instead of Ringer's solution. In spite of this loss of oxidizing power by diffusion the oxygen consumption of the fragments can be increased by previous lack of oxygen.

Oxygen consumption and irritability. Unwashed muscles not only have a higher resting oxygen consumption than washed muscles but they are peculiarly prone to show remarkable further increases in oxygen consumption either spontaneously or after anaerobiosis. The experiments illustrated in figure 4 are interesting in this connection. In figure 4a two match muscles were compared, one being washed in Ringer's solution and the other put into the respirometer without the use of Ringer's at all. The rate of oxygen consumption of the unwashed muscle was about twice that of the washed muscle for 4 hours when there was a sudden entirely spontaneous increase and in the space of one hour its rate became 4 to 5 times as high as that of the washed muscle. Meanwhile the unwashed muscle had become practically non-irritable while the washed muscle contracted normally. Some rather sudden irreversible change evidently took place in the unwashed muscle at the end of the first four hours for at the end of the experiment even a thorough soaking in Ringer's solution did not cause complete recovery of contractility Evidence will be presented below to show that this increase in oxygen consumption is sometimes at least associated with a gradual shortening of the muscle suggesting something akin to rigor. This experiment also shows that a similar rise in oxygen consumption may even occur in a washed muscle some time after dissection as in the experiment of Martin cited above.

The second experiment (b) of figure 4 is rather striking. Both muscles were unwashed at the start and when oxygen consumption measurements began the rate was very high in both and was definitely increasing. When tested for contractility 2 and 3 hours respectively after the beginning of the experiment both muscles were non-irritable. One of these muscles was then soaked in Ringer's solution for 15 minutes while the other was left unwashed as a control. Ringer's solution not only caused complete recovery of contractility but an enormous depression in the oxygen consumption from 6.0 to a normal rate of 0.8 cu. mm. per gram per minute. It is interesting that here as in figure 4a the oxygen consumption of the unwashed muscle passes through a high maximum and then slowly declines. Here also the change is more or less irreversible for soaking in Ringer's did not cause more than a partial recovery.

The third experiment (c) of figure 4 shows again an enormous rise in oxygen consumption following nitrogen. The recovery was followed for

8 hours and the oxygen rate was observed again to pass through a maximum after 4 to 5 hours and then gradually to fall. Both muscles in this case were non-irritable. They were not subsequently soaked in Ringer's solution to test their powers of recovery.

A further interesting feature is presented by figure 4d. Two match muscles, one washed (no. 9, table 2) and the other unwashed were put into hydrogen for 2.7 hours each. This caused no loss of contractility in the washed muscle but the oxygen consumption went very high in recovery and fell gradually to a low level characteristic of muscles in good condition. The oxygen regained was 6 times that which was missed. The unwashed

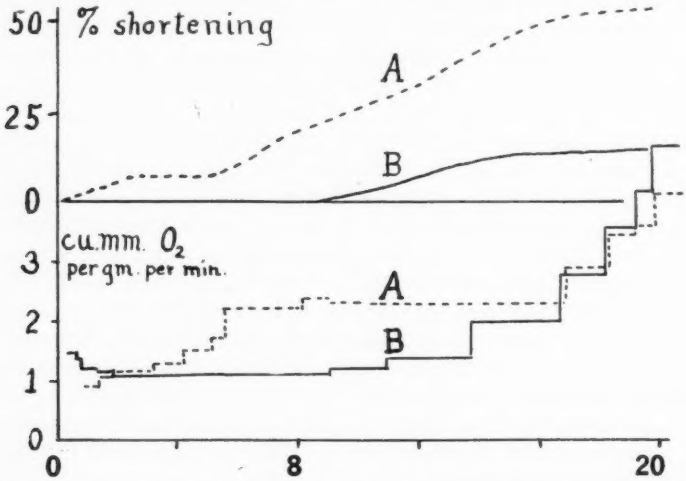

Fig. 5. Simultaneous observations of oxygen consumption and length changes in two unwashed semitendinosus muscles. Weights 157 (A) and 161 (B) mgm. 22.9°C. 30/0/20.

muscle on the other hand, although it had a higher oxygen rate before hydrogen had a lower rate than the control in the first 3 hours of recovery, although its basal rate remained higher. Possibly the anaerobic lactic acid mechanism does not function so well in non-irritable unwashed muscles.

These irreversible increases in oxygen consumption suggest a rigor mortis. Fletcher (1898) however in his study of the survival carbon dioxide output of frog muscles came to the conclusion that the shortening of rigor mortis took place without any increase in the gas exchange. To test this point small celluloid scales were placed inside the respirometers alongside which the muscles were hung sometimes with small brass weights hanging to them to keep them under slight tension. It was not difficult

to remove the respirometer momentarily from the water bath to measure the length of the muscles without interrupting the oxygen readings. The difficulty of these experiments lies in the uncertainty and delay in the onset of the shortening of rigor mortis

In the experiment of figure 5 oxygen consumption measurements were taken continuously for over 24 hours in two unwashed muscles, A and B. The oxygen consumption of A started to rise about 3 o'clock in the afternoon and more or less simultaneously it began to shorten. Likewise B began to shorten about midnight and simultaneously its oxygen consumption began to increase. The final abrupt rise in the oxygen consumption

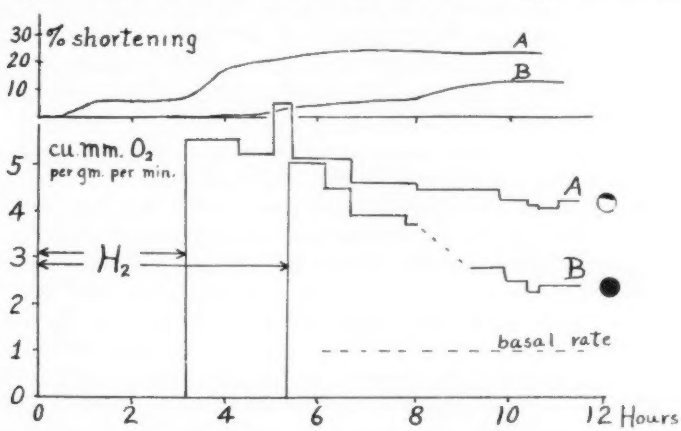

Fig. 6. Simultaneous observations of changes in length and oxygen consumption of two semitendinosus muscles after hydrogen. Weights 204 (A) and 197 (B) mgm. 22.9°C. 7/10/29.

may possibly be due to bacteria which could be observed in smears at this time.

In the experiments of figure 6 two unwashed muscles were put into hydrogen for 3 and 5 hours respectively to hasten the onset of rigor. Both began to shorten at about the time that the recovery began and muscle A which shortened 25 per cent of its length had a larger oxygen consumption throughout the experiment than B which only shortened 13 per cent of its length, in spite of the fact that B had been in hydrogen longer than A. Also it was noted that B, with the lower oxygen consumption remained irritable and in reasonably good condition while A was nearly completely inexcitable.

These somewhat preliminary experiments make it appear that there may be a connection between shortening of rigor mortis and an increased

oxygen consumption which Fletcher failed to observe. On the other hand I have observed a similar increase in oxygen consumption without a corresponding shortening and vice versa. It seems likely that the shortening which has been described as rigor mortis is only one of many manifestations of a fundamental structural disintegration throwing into confusion the chemical reactions of the muscle. It also appears that death of the muscle cell with its increase in permeability does not involve a cessation but rather a marked acceleration of the oxygen consumption (provided of course that oxygen is available).[2] Adolph (1929) has observed similar spontaneous increases in the oxygen consumption of surviving frog skin. It seems as if the smouldering fires were released at the death of the muscle and burst into flames. This process of cell death, however, involves undoubtedly a complicated series of reactions which probably occur in a different sequence in difference cells and at different times. Generalizations therefore should not be hastily made.

The condition of spontaneous non-irritability is very similar to that caused by soaking a muscle in an isotonic sugar solution. (Overton, 1902.) It is therefore a matter of considerable interest to find that sugar (dextrose, sucrose, maltose and lactose) also causes a marked increase in the oxygen[3] consumption which persists for at least 3 to 4 hours and sometimes longer. This similarity between spontaneous non-irritability and the non-irritability due to sugar would suggest that the former, like the latter, is due to loss of sodium ions by diffusion. However, an increased concentration of potassium ions in the intercellular spaces is another possible explanation which has been suggested by Duliere and Horton (1929) and by Hill (1929). In view of this theory it was of interest to find that solutions high in potassium concentration caused a similar increase in the rate of oxygen consumption of muscle. In this case however there is a considerable contraction of the muscle which seems to explain adequately the high oxygen consumption observed. Details of these experiments will be reported more fully at a later time. It is sufficient to point out at present that both isotonic sugar solutions and solutions high in potassium cause reversible loss of irritability and an increased oxygen consumption. These experiments therefore conform to the general rule that muscles in good condition have a low oxygen consumption and vice versa.

[2] It is possible if not probable that this behavior is peculiar to muscle. Osterhout (1922, p. 96) for example points out that CO_2 output decreases as the electrical resistance decreases or permeability increases with death in Laminaria.

[3] Since sending this paper to press I find that Embden and Lange, 1923 (Zeit. f. physiol. Chem., cxxv, 258) have described an increased oxygen consumption due to sugar and also increased loss of phosphate from muscles previous to rigor mortis. Certain of the other points in this paper are in agreement with recent heat production measurements of A. V. Hill, 1929, Proc. Roy. Soc. B, cv, 298 and of Hill and Kupalov, 1929, ibid., cv, 313.

SUMMARY

1. A muscle in good condition regains in oxygen about 70 per cent of the amount of oxygen which it missed during a previous anaerobic period.

2. Under certain as yet undefined conditions a muscle brought into oxygen after a period in nitrogen may consume an extra amount of oxygen which is 5 or more times as large as the oxygen missed during the nitrogen treatment.

3. Muscle as compared to nerve and other tissues possesses a peculiarly effective mechanism for surviving in nitrogen.

4. Muscles which become spontaneously non-irritable after dissection through failure to wash them in Ringer's solution have an oxygen consumption which averages 1.7 times the normal.

5. Muscles rendered non-irritable by soaking in isotonic glucose, sucrose, lactose or maltose or KCl solutions have likewise a high oxygen consumption.

6. Muscles, particularly when unwashed in Ringer's solution, are especially liable to show sudden increases in oxygen consumption. Such an increase in metabolic rate may be a manifestation of rigor mortis since it is, at times anyway, clearly associated with a mechanical shortening of the muscle.

7. As a general rule muscles in good condition have a low rate of oxygen consumption and vice versa.

I am much indebted to Mr. W. B. Latchford for invaluable assistance in the conduct of these experiments.

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THE ACTION OF HISTAMINE ON THE BRONCHIOLES AND PULMONARY VESSELS OF THE GUINEA PIG[1]

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Since the early work of Dale and his collaborators (1906, 1909, 1910, 1926) the action of histamine upon the bronchioles of guinea pigs has been known. Spasm of the bronchiolar muscles and consequent asphyxia are induced, and the lungs are found to be distended because the air cannot escape through the narrowed bronchi. Histamine has, also, a constrictor effect on the pulmonary arterioles, and the action may be so severe as to cause acute dilatation of the right side of the heart. This constriction has been found either by recording the pressure directly or by artificial perfusion of a surviving lung. Fühner and Starling (1913), working with a heart-lung preparation on dogs, obtained rises in the pulmonary pressure using doses of 0.5 mgm. of histamine. The only figures showing the action of histamine on the bronchioles of the guinea pig are those of Koessler and Lewis (1927), who obtained evidence of bronchoconstriction with intravenous injections of 0.005 mgm. of histamine, and those of Dale and Laidlaw (1910) who used 0.5 mgm.

A search of the literature fails to show whether or not these two phenomena, bronchoconstriction and pulmonary vasoconstriction, always occur together; or whether it is possible to get a rise in the pulmonary pressure without a corresponding bronchiolar constriction and *vice versa*. Nor are there data available indicating the sensitivity of the bronchioles and pulmonary vessels to injections of histamine. With any methods utilizing the intact animal, there is always difficulty in deciding whether reactions taken to indicate bronchiolar constriction are not due to changes in pulmonary blood flow, pressure, or volume, and the methods employed in these experiments are not entirely free from these sources of error. Similarly it is conceivable that air trapped in the alveoli by bronchoconstriction might cause obstruction to pulmonary blood flow and a consequent rise in pulmonary arterial pressure.

METHODS. 1. *Bronchiolar constriction.* A glass body-plethysmograph into which the guinea pig could be placed up to the neck was employed,

[1] The expenses of this investigation were met in part by an anonymous donation known as the "M. G. H. Asthma Fund."

and from this the respiratory movements were recorded on a kymograph by means of a small Brodie bellows. The right carotid artery was tied off and the animal decerebrated under ether anesthesia, the whole operation taking around seven minutes. The animal was allowed to come out of the ether and to recover completely from shock before being placed in the plethysmograph. Injections were made through the exposed jugular vein, not earlier than thirty minutes after the completion of the operation.

Due to the fact that solutions of histamine deteriorate upon standing, none used in any of these experiments was more than twenty-four hours old. The average length of the period of injection was nine seconds and in all cases 0.5 cc. of liquid was introduced. When an animal so treated reacts by bronchoconstriction to histamine the entire curve of respiration recorded by the Brodie bellows rises markedly, indicating a swelling of the thorax which expresses the inability of the animal to expel all the air inhaled.

The second method employed to determine the onset of bronchiolar obstruction was the air-overflow method, previously described by Went and Drinker (1929). In this method a T-tube is placed on the inflow line from an artificial respiration pump to the tracheal cannula. The end of the T-tube is just immersed in mercury in a small bottle. A second shorter tube leads from the bottle to a small Krogh spirometer which writes upon a kymograph (see fig. 1). Animals so employed were all decerebrated and then given curare and artificial respiration. When bronchoconstriction occurs, the entrance of air into the lungs is made more difficult and air overflows into the Krogh spirometer which records this overflow upon a kymograph.

2. *Pulmonary vasoconstriction.* The action of histamine on the pulmonary vessels was measured in accordance with a technique briefly described by Drinker and Went (1928, 1929). Because of the applicability of this method to many types of physiological experiments, we take the liberty of describing it here in greater detail. A small L-shaped glass cannula was passed into the arch of the pulmonary artery without in any way obstructing the pulmonary blood flow. The cannula was connected to a water manometer colored with methylene blue. An intravenous injection of heparin was given to prevent blood coagulation. The recording of the manometer level was entirely automatic, use being made of a Bell-Howell 16 mm. motion picture camera, equipped with a small solenoid operating a plunger, and arranged to strike the starting button of the camera. The current in the solenoid was made and broken at regular intervals by the use of an ordinary laboratory timer, thus providing photographic records of the height of the manometer. A clock was included in the photographic field, giving the times essential for plotting the pulmonary pressure curve of the experiment. By means of this apparatus, it is

possible to take as many as sixteen pictures a second, though practically, pictures taken at intervals of one or two seconds recorded the experiment in sufficient detail. (See figs. 1 and 2.)

RESULTS. 1. *Bronchoconstriction by body-plethysmograph.* Koessler and Lewis (1927) have observed bronchoconstriction in guinea pigs using doses of 0.005 mgm. of histamine per animal. In view of the results which

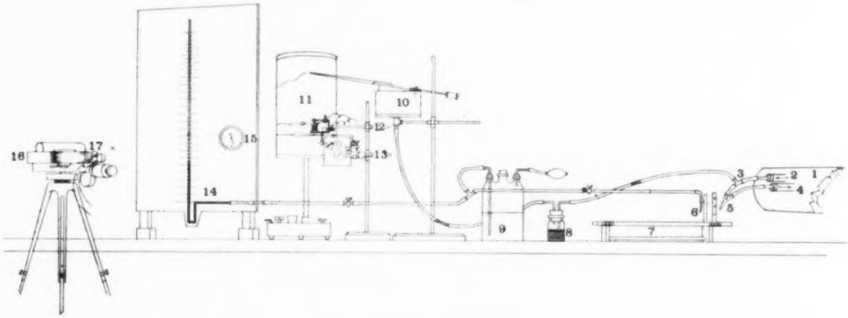

Fig. 1. Apparatus for measuring pulmonary arterial pressure and bronchoconstriction in the guinea pig. *1*, artificial respiration pump; *2*, inflow line; *3*, T-tube; *4*, outflow line; *5*, tracheal cannula; *6*, pulmonary arterial cannula; *7*, animal board; *8*, mercury bottle; *9*, pressure bottle filled with saline; *10*, Krogh spirometer; *11*, kymograph; *12*, signal magnet; *13*, Jaquet clock; *14*, water manometer; *15*, clock; *16*, Bell-Howell motion picture camera; *17*, solenoid.

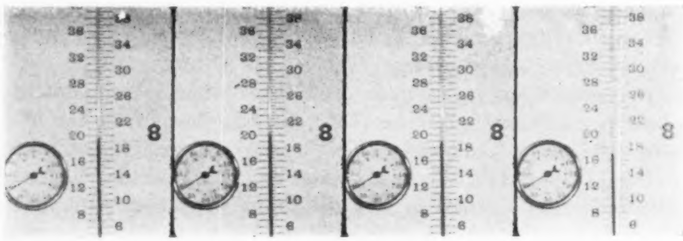

Fig. 2. Photographic record of manometer. Individual pictures one second apart. Manometer recording pulmonary arterial pressure.

we have obtained with the body-plethysmograph, this appears to be a fairly large dose. Table 1 gives the minimal effective dose of histamine in 21 different animals.

It will be noted that in every animal, with the exception of one, we were able to detect bronchoconstriction with doses of 0.004 mgm. or smaller. Twelve of the animals responded to doses between 0.0001 and 0.0008 mgm. which represent doses between 0.00028 and 0.00229 mgm. per kilo

TABLE 1

Bronchiolar constriction from histamine, body-plethysmograph method

NUMBER	DATE	WEIGHT	MINIMAL EFFECTIVE DOSE	DOSE PER KILOGRAM
	1929	*grams*	*mgm.*	*mgm.*
1	July 29	385	0.00020	0.00052
2	July 30	400	0.01500	0.03700
3	August 1	350	0.00010	0.00028
4	August 1	375	0.00080	0.00213
5	August 2	355	0.00080	0.00220
6	August 2	350	0.00080	0.00229
7	August 3	370	0.00080	0.00216
8	August 5	380	0.00080	0.00210
9	August 5	380	0.00020	0.00052
10	August 6	447	0.00070	0.00156
11	August 6	420	0.00100	0.00238
12	August 7	385	0.00004	0.00010
13	August 8	405	0.00100	0.00247
14	August 9	340	0.00400	0.01170
15	August 14	355	0.00080	0.00220
16	August 15	235	0.00050	0.00212
17	August 19	323	0.00030	0.00092
18	August 26	380	0.00100	0.00260
19	August 28	350	0.00200	0.00571
20	August 29	380	0.00200	0.00526
21	August 29	380	0.00100	0.00263

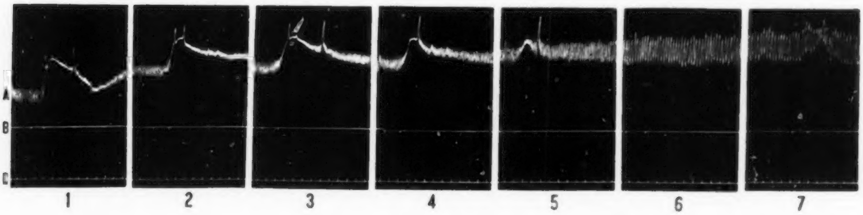

Fig. 3. Bronchiolar constriction from histamine, body-plethysmograph method. *A*, respiration; *B*, period of injection; *C*, time, at intervals of 6 seconds. Between the two marks on *B*, section *1*, 0.0008 mgm. of histamine were injected intravenously. The reaction observed is greater in degree than those which follow with decreasing amounts of histamine. Section *2* shows the effect of a dose of 0.006 mgm. of histamine; section *3*, of 0.004 mgm.; section *4*, of 0.002 mgm.; section *5*, of 0.001 mgm.; section *6*, of 0.3 cc. of normal horse serum over one year old; and section *7*, of 0.0008 mgm. of histamine. The rise in the respiration curve, indicating the increasing inability of the animal to expel all of the air inhaled, should be noted.

of body weight. A typical record is shown in figure 3. The highest sensitivity observed occurred with a dose of 0.00004 mgm. or equal to 0.0001 mgm. per kilo.

TABLE 2

Bronchiolar constriction from histamine, air-overflow method

NUMBER	WEIGHT	DOSE	BRONCHOCONSTRICTION	DOSE PER KILOGRAM
	grams	*mgm.*		*mgm.*
1	510	0.0010	++	0.00196
2	490	0.0020	++	0.00400
3	515	0.0008	++	0.00156
4	710	0.0005	++	0.00070
5	485	0.0005	++	0.00100

TABLE 3

Pulmonary pressure rise without bronchiolar constriction

NUMBER	WEIGHT	DOSE	PRESSURE	BRONCHOCONSTRICTION	DOSE PER KILOGRAM
	grams	*mgm.*			*mgm.*
1	460	0.00060	++	–	0.001300
2	425	0.00010	+	–	0.000230
5	640	0.00100	++	–	0.001500
	640	0.00200	+++	–	0.003100
7	430	0.00004	++	–	0.000093

TABLE 4

Pulmonary pressure rise with bronchiolar constriction

NUMBER	WEIGHT	DOSE	PRESSURE	BRONCHOCONSTRICTION	DOSE PER KILOGRAM
	grams	*mgm.*			*mgm.*
1	460	0.0010	+++	+++	0.00210
2	425	0.0010	+++	+++	0.00230
3	550	0.0040	+++	+++	0.00720
	550	0.0060	+++	+++	0.01000
5	640	0.0040	+++	+++	0.00620
	640	0.0032	+++	+++	0.00500
7	430	0.0005	+++	+++	0.00110
	430	0.0001	++	++	0.00023

2. *Bronchoconstriction, air-overflow method.* Table 2 shows the results obtained by the use of the air-overflow method. A smaller series of animals was used, but the results are in agreement with those obtained with the body-plethysmograph. This method is probably quite as sensitive as the former.

3. *Bronchoconstriction and pulmonary pressure.* Doses of the same magnitude that cause a bronchiolar constriction, as determined by the use of the plethysmograph and the air-overflow methods, are effective in causing a rise in pulmonary arterial pressure. It is possible, in a given animal, with a small dose of histamine, to get a rise in pulmonary pressure without any accompanying bronchoconstriction, but with slightly larger doses the two phenomena invariably occur together. (See tables 3 and 4.) This may be partially due to a difference in the sensitivity of the two methods though, as table 2 shows, we have been able in some animals to detect bronchoconstriction with doses smaller than some of those which caused only pulmonary vasoconstriction. On the other hand, we have never been able to get a bronchiolar effect without good evidence of

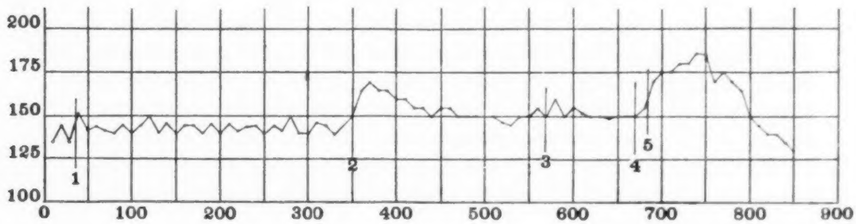

Fig. 4. Pulmonary arterial pressure in a normal guinea pig. Ordinates, pressure in millimeters of water. Abscissae, time in seconds. At mark *1*, 0.000,04 mgm. of histamine was injected intravenously; at mark *2*, 0.0001 mgm.; at mark *3*, 0.5 cc. of physiological saline; at mark *4*, 0.001 mgm. of histamine. The onset of bronchiolar constriction occurred at mark *5*.

constriction in the pulmonary vessels. Figure 4 shows a typical experiment, the protocol of which is as follows:

October 10, 1929. *Experiment 2. Intravenous injection of varying doses of histamine.* Normal guinea pig. Weight 425 grams. 9:45 a.m., ether. 10:00 a.m., carotids tied off, decerebration completed. 11:35 a.m., cannulation of external jugular vein and pulmonary artery finished. Animal curarized, heparinized, and under artificial respiration with connection to Krogh spirometer. Pressure record begun as shown in figure 4. At mark *1*, 0.000, 04 mgm. of histamine was given intravenously, with no change in the pressure. At mark *2*, 0.0001 mgm. of histamine was given intravenously. There was an immediate rise in the pulmonary pressure, but no evidence of bronchiolar constriction. At mark *3*, 0.5 cc. of physiological saline was given intravenously. The slight rise in pressure indicates the possible degree of volume effect. At mark *4*, 0.001 mgm. of histamine was given, with an immediate increase in pulmonary pressure and bronchoconstriction at mark *5*.

An experiment of this type, with such definite effects on pulmonary arterial pressure and without indication of change in the difficulty of

entrance of air until large doses of histamine are used, seems explainable only on the basis of a pure constrictor effect upon the pulmonary vessels. It is well known that the most frequent cause of rise in the pulmonary blood pressure is an increased filling of the right ventricle. There is nothing in the action of histamine which would cause us to believe this sort of effect has anything to do with the pressure rises which have been observed. Furthermore, if one injects 5 cc. of heparinized blood into the jugular vein of a guinea pig, prepared for measurement of obstructed air entrance by the method which has been described, air overflow begins at once. This means that given an efficient heart, it requires very slight increase in pulmonary blood volume to exclude air. Every action of histamine would seem against raising pulmonary pressure and excluding air through any such pulmonary blood volume change.

Furthermore, in the air-overflow preparation, arrangements are such that air pressure within the alveoli cannot increase and so permit alveolar distention to be a factor in causing a rise in pulmonary arterial pressure. It is thus safe to conclude that rises in pulmonary blood pressure due to histamine injections are unexplainable save by the independent effect of histamine on the pulmonary blood vessels, and that the possibility of secondary pressure effects due to bronchoconstriction is not a necessary part of such reactions.

SUMMARY

1. The experiments cited show that the two effects—pulmonary vasoconstriction and bronchoconstriction—are not readily separated except in the case of very small doses of histamine, with which it is possible to get a rise in pulmonary pressure without any evidence of bronchoconstriction. The possibility of a difference in the sensitivity of the methods employed for detecting these two phenomena must be taken into account but, for reasons already given, does not negative this conclusion.

2. Bronchoconstriction was obtained in guinea pigs with doses of histamine ranging from 0.000,10 mgm. to 0.011,70 mgm. per kilo of body weight, 25 of the 33 animals studied responding to doses ranging from 0.000,10 mgm. to 0.002,63 mgm. per kilo of body weight.

3. Pulmonary vasoconstriction, without evidence of bronchoconstriction, was obtained with doses of histamine ranging from 0.000,093 mgm. per kilo of body weight to 0.003,10 mgm. per kilo of body weight. In this same series of animals, bronchoconstriction was obtained, in addition, only with larger doses, ranging from 0.000,23 mgm. to 0.10 mgm. per kilo of body weight.

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STUDIES ON THE PHYSIOLOGICAL ACTION OF LIGHT

VIII. An Attempt to Characterize the Substance Giving Increased Uric Acid Values After Irradiation of Blood[1]

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In an earlier publication, Koch and Reed (1926) reported that direct irradiation by carbon arc lamp of blood flowing through a quartz tube inserted into the carotid artery in etherized dogs, produced an apparent increase in the uric acid content, as determined by the phosphotungstate colorimetric method. However, it was recognized at the time that there was no actual increase in uric acid, but that the reaction was due to some other reducing substance. It was thought that dioxyphenylalanine might be responsible on account of its supposed relation to pigmentation (Bang, 1924).

In the present investigation, an attempt was made to characterize the substance, or *substances*, which appear to be augmented as a result of irradiation of blood. Employing the same technic as in the earlier experiments, except that in most experiments a water-cooled Kromayer lamp was used as a source of radiation, a series of experiments was made in which uric acid values were obtained by the method of Folin (1922). In those experiments wherein an increased reduction was observed, an attempt was made to identify dioxyphenylalanine or a closely related derivative by a modified Comessati reaction.[2] In several experiments

[1] This investigation was financed in part by grants from the Committee on Scientific Research of the American Medical Association and from the Phi Rho Sigma Medical Fraternity.

[2] The modified Comessati reaction developed for this purpose utilized potassium ferricyanide in sodium borate as the oxidizing reagent. With Mulford's epinephrin solution, 1-1000, accepting the manufacturer's standardization as approximately correct, four equivalents of ferricyanide produced maximum pink color formation. The color produced by less ferricyanide was directly proportional to the maximum color production with four equivalents. To determine the amount of adrenalin, any two ratios of ferricyanide: adrenalin less than 4:1 would suffice to establish a straight line of color intensity, this line cutting the horizontal (maximum color intensity) at $\frac{\text{(M) ferricyanide}}{\text{(M) adrenalin}} = 4$. The pressor effect of adrenalin was found to be completely destroyed when four equivalents of ferricyanide were added, but not with less than

determinations of blood phenols were made by the method of Theiss and Benedict (1924). Since the substances which give the Folin uric acid reaction and those reacting with the Theiss-Benedict reagent are so widely diverse, it was thought unprofitable, for the present, to express the results in terms of concentration of any one substance. Therefore the color production in each instance before irradiation was used as a standard and any changes in the colorimetric values in the blood after irradiation were expressed in percentage.

In table 1 are shown the results of nine experiments. In five experiments there was an increase ranging from 6 per cent to 100 per cent with an average of 31 per cent in reducing power. A similar range of increase was noted by Koch and Reed, with an average increase of 38 per cent. In three experiments there was a decrease, with no change in one case. In four of the five experiments in which phenols were determined simultaneously, there was a definite decrease of the concentration of the latter. The significance of this divergence will be investigated later. In no instance was there a positive Comessati reaction, consequently it appears that neither dioxyphenylalanine nor any related compound is involved in the reaction.

TABLE 1

EXPERIMENT NUMBER	PERCENTAGE CHANGE IN COLOR INTENSITY			IRRADIATION TIME	PHENOL (THEISS-BENEDICT) PER CENT CHANGE
	+	−	0		
318				2 hours	
319	15			1 hour 30 minutes	
323	100			1 hour 40 minutes	
324	18.4			1 hour 40 minutes	+27.5
325		28.6		1 hour	−17.5
326	6.1			1 hour 30 minutes	−2.2
327	15.7			1 hour 30 minutes	
329		11		1 hour 40 minutes	−39.5
328		Koch 31.3 Folin 56.0		1 hour 10 minutes	−16

In experiment 328, both the Folin method and Koch's modification were used on the same samples, from which it appears that Folin's method is the more sensitive.

In order to determine whether this reaction could be produced in vitro,

four. Electrometrically, it was found that this ferricyanide method had an undeterminable error due to reduction of ferricyanide greater than 4 equivalents, the excess apparently being reduced by the aromatic nucleus, since it was found that the rate of reduction of ferricyanide, followed potentiometrically, took place according to the autolytic curve.

six experiments were performed on samples of fresh whole blood. In four of these, the blood was placed in a flat quartz flask, approximately 5 mm. inside diameter, which was placed directly against the window of the Kromayer lamp. In two other experiments, the flask was placed at a distance of one foot from a carbon arc lamp, similar to that employed in the earlier experiments.[3] In the latter experiments, there were increases of 3 per cent and 12.5 per cent respectively. In the other four the increase ranged from 2.5 per cent to 66 per cent. Heparinized plasma, obtained from these samples, was also irradiated, resulting in a decrease of small magnitude. Solutions of hemoglobin and of albumen failed to give any reaction after 1 hour of irradiation with either lamp.

In the experiment in which there was a 66 per cent increase after irradiation, the irradiated sample was allowed to stand over night at room temperature. It now failed to give any reaction with the Folin reagent, even after a second irradiation. This sugested glucolysis; so it was determined

TABLE 2

A sample of dog blood was divided into two portions, A and B. Each was irradiated 30 minutes with the Kromayer lamp

	PERCENTAGE INCREASE IN REDUCING POWER
A + = Volume Ringer solution	4
B + = Volume Ringer solution with 1 per cent dextrose Final concentration 0.55 per cent	14.8

to study the effects of irradiation on solutions of dextrose and of blood to which dextrose had been added.

That dextrose is decomposed by irradiation has been reported by several investigators (Neuberg, 1908, 1910; P. Mayer, 1911; Berthelot and Gaudechon, 1910; Bierry, Henri, and Ranc, 1911; Lippmann and Völker, 1928; Pincusson, 1922; E. Mayer, 1927). The final products of decomposition were carbon monoxide, carbon dioxide, and water. Intermediate products were various alcohols, aldehydes, and ketones, many of which have reducing power of greater magnitude than dextrose itself; e.g., they will reduce Fehling's solution in the cold. In fact dextrose will reduce phosphotungstic acid to some extent and blood dextrose is one of the most serious sources of error in the direct determination of blood uric acid. And it has been found that irradiation for periods up to two hours augmented this power. But if the irradiation is continued long enough, depending on the concentration, the reducing power disappears completely.

[3] Loaned by the Paul E. Johnson Co. for experimental purposes.

Acetaldehyde and dihydroxyacetone, two of the possible intermediate substances found in the decomposition of dextrose by ultraviolet rays, were found to give pronounced reduction of phosphotungstic acid in the presence of cyanide, particularly the latter, which does not reduce when cold unless cyanide is present, in which case reduction occurs immediately.

In two experiments in which 0.1 per cent dextrose solution was irradiated for 1 hour in each, the increases in reducing power were 22.5 per cent and 27.9 per cent respectively. None of the irradiated dextrose solutions gave a positive phenol reaction.

In table 2 are shown the results of an experiment on fresh whole blood. From this it is apparent that dextrose plays an important part in the reaction although the possibility is not excluded that other normal blood constituents may participate.

Another sample of fresh whole dog blood was divided into two portions, one of which was irradiated in a quartz flask, the other in a glass flask of the same dimensions. After one hour of irradiation the contents of the quartz flask showed an increase of 16 per cent, while those of the glass flask showed an increase of 28 per cent.

A suspension of washed erythrocytes in Ringer solution showed an increase at the end of one hour of irradiation of only 4.5 per cent; at the end of one and one-half hours, 7 per cent increase. The plasma obtained from whole blood showed a decrease of 2 per cent. Apparently decomposition was more rapid in these cases.

Quantitative determinations of blood sugar were not made in any of these experiments. Correlation of this factor with the reducing power after irradiation may possibly offer some clue to the variability noted in these experiments and those of Koch and Reed. This point will be a subject for future investigations.

Solutions of uric acid were found to lose their reducing power after irradiation, but the effect of the presence of blood on the rate of loss has not yet been studied in detail. This may still be a factor in the variations apparent in these experiments. It is also possible that the decomposition of dextrose in blood may be modified by substances acting as photocatalysers; still further, there may be elimination of the more volatile products of sugar decomposition, at variable rates, depending on the condition of individual animals. To what extent blood sugar concentration is decreased to produce the increased reduction of Folin's reagent remains to be determined. Reed, Payte, and Lackey (1926) failed to find any constant effect of carbon arc irradiation on the blood sugar concentration of etherized dogs, using the same technic as employed in all the earlier experiments. However, numerous investigators have reported a decrease from irradiation of unanesthetized subjects. Pincusson (1922) found an increased reducing power, but a decrease in concentration of blood sugar in

rabbits, and attributed his results to two factors, increased mobilization and increased oxidation. Similar results have been reported by Rothman (1924), Bloch and Faber (1925), and by Ferri (1927) in clinical patients. Pincusson and Kawakami (1929) believed that irradiation acts like insulin. On the other hand, Hall and Root (1928) reported an increase in blood sugar concentration in irradiated rabbits, even through glass, and suggested several possible explanations of the results.

The failure of Reed, Payte, and Lackey to obtain constant results may possibly have been due to the anesthetic. However, since the blood was irradiated directly and the skin was not subjected to irradiation at all, it is possible that this may be a factor. The recent work of Folin, Trimble, and Newman (1927) on the function of the skin as a dextrose storage tissue renders this possibility of more importance.

Whether the decomposition products formed in dextrose solutions have any influence on blood pressure is under investigation at present and is of importance in view of the profound depression of blood pressure produced by direct irradiation of blood in vivo (Reed, 1925).

SUMMARY

1. Direct irradiation of blood in vivo in etherized dogs by means of a Kromayer water-cooled lamp or by carbon arc, usually produces an increase in the phosphotungstic acid reducing power of blood filtrates. For convenience the factor responsible for this increased reducing power is considered as one substance, although it is recognized that more than one substance may be involved.

2. The increase is not due to uric acid, since this is decomposed by irradiation and a negative Comessati reaction shows the absence of dioxyphenylalanine.

3. Similar reactions are obtained with fresh whole blood when irradiated in vitro.

4. Solutions of dextrose, when irradiated, show a similar range of increase in reducing power.

5. When blood to which dextrose has been added is irradiated in vitro, the reducing power is augmented.

6. Apparently decomposition of blood sugar is the most important factor in the production of this reaction.

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BLOOD SUGAR OF ADRENALECTOMIZED RATS

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This study was undertaken to determine the amount of sugar in the blood of adrenalectomized rats in the quiet state and under excitement (anger and pain). For comparative purposes similar determinations were made on a group of normal rats.

Boggild (1925) found a decided lowering of the blood sugar level in rats after adrenalectomy. Sundberg (1925) with adrenalectomized rabbits and Rogoff and Stewart (1926) with suprarenalectomized dogs give the blood sugar content as being normal. Wyman and Walker (1929) found hypoglycemia in adrenalectomized rats and review this phase of the literature.

As to the production of hyperglycemia, Griffith (1923) showed very definitely that this occurs after stimulation of various nerves, as the sciatic, crural or vagus in adrenalectomized cats under chloralose anesthesia. Stewart and Rogoff (1917) obtained hyperglycemia from piqûre and asphyxia in adrenalectomized dogs. Hirayama (1926) found that as a result of tying or restraining adrenalectomized rabbits, the ensuing struggling and excitement caused an increase in the blood sugar concentration in three out of seven cases.

Methods. Our rats had both adrenals removed at one operation using the method of Lewis (1923). Seven days were allowed for recovery before any sugar determinations were made.

In these experiments the blood sugar of the rats in the quiet state after being given ether carefully, was determined, then approximately one hour later the same animal was fastened to an animal holder and teased by being stimulated from time to time for a period of ten minutes with an interrupted Faradic current by means of a Harvard inductorium. As quickly as possible after this treatment a second blood sugar determination was made.

For the sugar determinations we used the method of Folin (1928) slightly modified. The blood was obtained under ether anesthesia from the heart by means of an hypodermic syringe. Eight drops of this blood were delivered into each of two weighed centrifuge tubes containing tungstic acid solution and their weight again obtained. The eight drops weighed

approximately 75 mgm. This is somewhat less than the 0.1 cc. as called for by Folin, but was found suitable for color comparisons. From this point the routine of the Folin method was followed. Double determinations were always made. The averages of these determinations in milligrams per one hundred grams of blood are given in the tables.

TABLE 1

Blood sugar in normal rats

NUMBER	QUIET	EXCITED	PER CENT INCREASE
	Males		
77	130	205	57.7
72	153	187	22.2
20	134	177	32.1
12	147	275	87.1
12	162		
80	125	236	88.8
80	165		
Average..........	145	216	48.3
	Females		
91	145	162	11.7
81	129	154	25.7
67	124	156	25.8
Average..........	133	157	18

TABLE 2

Blood sugar in adrenalectomized male rats

NUMBER	QUIET	DAYS AFTER OPERATION	EXCITED	DAYS AFTER OPERATION	PER CENT OF CHANGE
90	114	7	125	7	+9.7
98	110	7	111	7	0
A3	124	7	160	7	+27.6
A3	93	8	75	8	−19.3
A7	131	9	77	9	−41.2
A9	130	9	117	9	−10.0
A10	142	8	137	8	−3.5
A12	179	7	161	7	−10
Average....	127.9		120.4		−5.5

RESULTS. Table 1 is a record of the blood sugar in quiet and excited normal male and female rats. After stimulation there is a decided in-

crease in the blood sugar of both groups but that in the males is considerably higher than in the females. There is an average increase of 48.3 per cent in the males and 18 per cent in the females.

The effect on the blood sugar in adrenalectomized male rats is shown in table 2. In this group the blood sugar content is lower in both the quiet and excited states than in the normals. In two cases there is an increase in the excited stage, in five a decrease and in one no change. The average after excitement shows a slight decrease, viz.: for the quiet state 127.9; after excitation 120.4. The range in the quiet state was 93 to 179; when excited 75 to 161.

Eight determinations on five adrenalectomized females in the quiet state gave an average of 112 with a range of 92 to 124; while 8 determinations in the excited state averaged 100 with a range of 80 to 125. Here again we find a somewhat lower blood sugar level in the females than in the males. There was no increase after excitement.

Discussion. In these experiments ether was used as an anesthetic for obtaining the blood. Scott (1914) found that ether anesthesia increases the blood sugar. This fact may have caused our basal levels to be higher than would have been if blood had been obtained in the quiet state without an anesthetic. We found it impracticable to obtain a sufficient amount of blood from these rodents for our determinations without anesthesia because of exciting their anger.

Most investigators report an increase in blood sugar in some of their adrenalectomized animals after strong stimulation, but these increases are less certain and less pronounced than in normal subjects. We found adrenalectomized rats less excitable than normal animals and the blood sugar changes irregular and inconsistent.

From these experiments it seems safe to conclude that the adrenal glands play an important rôle in affecting sugar mobilization.

SUMMARY

1. Normal rats exhibit a well defined hyperglycemia on being tied down and stimulated with an interrupted Faradic current. This condition was more marked in males than in females.

2. After stimulation the adrenalectomized rats showed on the average no increase in their blood sugar, although they displayed the outward signs of excitement and rage, such as biting and struggling.

3. The blood sugar in the adrenalectomized rats was lower on the average in the quiet state than in a similar condition in the controls.

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THE INFLUENCE OF INORGANIC IRON ON THE ANEMIA OF RICE DISEASE IN PIGEONS

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The use of iron in the nutritional types of experimental anemias has given rise to rather contradictory results depending on the character of the deficiency and the severity of the anemia produced. A favorable influence of $FeCl_3$ on the rate of blood regeneration of rats rendered anemic by a bread and milk diet has been reported by Scott (1924). Mitchell and Schmidt (1926) and Mitchell and Vaughn (1927) have reported similar results. McGowan and Crichton (1924) also observed that the severe anemia in swine on a milk diet was relieved by the addition of ferric oxide to the diet. Hart et al. (1927) noted favorable effects of both iron and copper on the blood regeneration of milk anemia in rats, but more recently these authors (Waddell et al., 1929) have ascribed the beneficial effects previously noted to traces of copper with which the iron was contaminated. Beard and Myers (1929), on the other hand, have noted a satisfactory blood regeneration with milk-anemic young rats following the administration of pure $FeCl_3$ and attributed the beneficial action directly to the Fe ion, although additions of traces of copper, nickel, cobalt or germanium to the iron produced better recoveries than the iron alone.

In the hemorrhage type of anemia of short duration in dogs or rats the influence of inorganic iron is negligible (Williamson and Ets, 1925), although in severe anemias over long periods of time, a definite blood regeneration following iron administration was observed by Hooper, Robscheit-Robbins and Whipple (1920), and Robscheit-Robbins and Whipple (1927). It would seem, therefore, that inorganic iron is utilizable for blood regeneration in experimentally induced anemias only under conditions where a dietary or actual body iron deficit exists.

An exclusive diet of polished rice fed to either pigeons or rats over a four to eight week period results in a typical inanition and the development of a more or less severe anemia. This diet, however, is deficient in vitamins A, B, and C in addition to being practically iron-free. The latter deficiency is thus one of the requirements for the development of the milk type of anemia. The addition of vitamins A and C to the rice diet did not

influence the course of the anemia (Barlow, 1928) as might be expected since Sugiura and Benedict (1923) have shown that pigeons do not require these vitamins for maintenance. The present study is a report on the effects of supplying one of the two remaining deficiencies of the polished rice diet, i.e., iron. Such a procedure also affords an opportunity for further comparing the phenomena of fasting and rice disease.

METHODS. Normal adult pigeons were used in all experiments. The observations taken weekly included the body weights, red cell counts, hemoglobin in grams per 100 ccm. of blood (Newcomer), and cell volumes (Van Allen hematocrit).

Six series of experiments (each of which included 9 to 22 birds) were made. All pigeons except the normal control series were placed on a polished rice diet for a three week period in order to develop a mild anemia. The blood regenerating efficiency of $FeCO_3$, added daily to the polished rice diets in dosages ranging from 2 to 200 mgm. per kilogram body weight, was then observed during the succeeding six weeks. Two types of control experiments were also included. One series received polished rice only, while the other received daily a normal diet throughout the period of study plus the maximal iron dosage. The latter experiment was deemed necessary in order to interpret any changes produced by the massive dosage (200 mgm. per kgm.) of iron on the course of the anemia of rice disease, since Williamson and Ets (1925) have reported that iron is toxic when given in large dosages.

The Blaud's pills in which form the $FeCO_3$ was administered were prepared weekly. The iron content of the pills was gauged to the dosage administered daily and the weight of the pigeons. Since the average pigeon weight was 300 grams, each pill contained either 0.65, 6.6 or 66.6 mgm. of $FeCO_3$ (corresponding to 1, 10 and 100 times the therapeutic human per kgm. body weight dosage).

RESULTS. *Therapeutic dosages.* Two series of pigeons (C and D) including 12 birds each were placed on a rice diet for a three week period. From the 3rd to the 8th weeks, 150 mgm. of Harris yeast extract was added to the rice diet of each bird in order to maintain the state of inanition and the degree of anemia at approximately 75 per cent normal. From the 8th to the 14th weeks group C received a therapeutic dose of $FeCO_3$ (0.65 mgm.) daily in addition to the rice and vitamin B, while group D received rice and a similar therapeutic dosage of iron without the vitamin B.

During the first eight weeks of the experiments the weight, cell counts, hemoglobin values and cell volumes of these two groups of birds showed no significant differences, (figs. 1, 2, 3 and 4). Group C on the addition of a therapeutic dose of iron was not influenced in any way during the ensuing period of study. The substitution of a therapeutic dosage of iron

for the vitamin B content of the diet of group D was followed immediately by the rapid development of rice inanition and the terminal values for the several observations corresponded very closely with those of the rice control series B. Therefore the administration of therapeutic dosages of iron did not counteract in any degree the anemia resulting from the inanition of partial or complete vitamin B deficiency.

The influence of large dosages of $FeCO_3$ on the blood picture. The failure of therapeutic doses (2 mgm. per kgm.) of iron to promote body weight or blood regeneration when added to the rice diets of groups C and D was also observed under similar conditions following addition of 10 (20 mgm. per kgm.) and 100 times (200 mgm. per kgm.) the therapeutic dosages of iron to the rice diets of groups E and F. These observations with $FeCO_3$ therefore confirm the results reported by Banejie (1927) on the ineffectiveness of Fe_2O_3 on the avitaminosis of pigeons.

The degree of anemia observed in group F (figs. 2, 3, and 4) which received the larger iron dosage was even greater than was noted with the iron free rice control series B. It appeared therefore that this dosage actually accelerated the rate of blood destruction in the pigeon as previously reported by Williamson and Ets for the dog. This was confirmed by the reactions of group A which received throughout the period of study, a normal grain diet to which the same dosage of $FeCO_3$ which was administered to group F, was added. The weight changes of group A (fig. 1) were due in part to a diminished food intake and perhaps to a local action of the iron on the alimentary tract. The blood changes noted in figures 2, 3 and 4 were apparently due to the effects of the absorbed iron (Williamson, 1925) on the hematopoietic organs since in addition to the anemia present, many bizarre red cell forms in addition to a strong anisocytosis was noted.

Figs. 1, 2, 3 and 4 represent the median changes in weight, hemoglobin, red cell counts and cell volumes of six series of pigeons (illustrated by the letters) while receiving the following diets:

Group A—Normal (whole mixed grain) diet plus 200 mgm. $FeCO_3$ per kgm. daily.

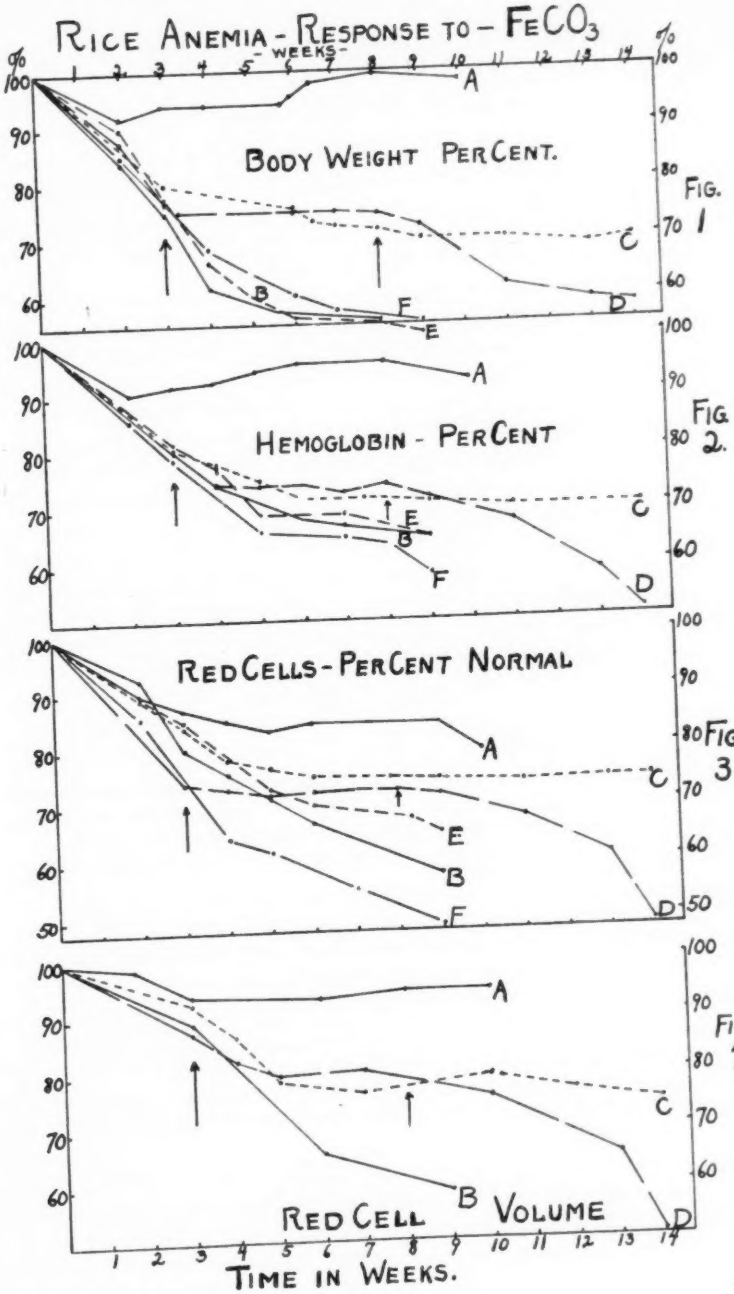
Group B—Polished rice only.

Group C—Polished rice from the 1st to 3rd weeks; rice plus 150 mgm. Harris yeast extract daily from the 3rd to 8th weeks, and rice plus 150 mgm. yeast extract plus 2 mgm. $FeCO_3$ per kgm. daily thereafter.

Group D received rice from the 1st to 3rd weeks, rice plus 150 mgm. yeast extract 3rd to 8th weeks and rice plus 2 mgm. $FeCO_3$ per kgm. daily from the 8th to 14th weeks.

Group E—Polished rice from the 1st to 3rd weeks and rice plus 20 mgm. $FeCO_3$ per kgm. daily from the 3rd to 9th weeks.

Group F—Polished rice from the 1st to 3rd weeks and rice plus 200 mgm. $FeCO_3$ per kgm. daily from the 3rd to 9th weeks.


RICE ANEMIA - RESPONSE TO - $FeCO_3$
- WEEKS -
BODY WEIGHT PER CENT.
FIG. 1
HEMOGLOBIN - PER CENT
FIG. 2.
RED CELLS - PER CENT NORMAL
FIG. 3
FIG. 4
RED CELL VOLUME
TIME IN WEEKS.

Figs. 1-4

SUMMARY AND CONCLUSIONS

Addition of ferrous carbonate as Blaud's pills during a six week period, in quantities ranging from therapeutic to toxic dosages, to the practically iron free rice diet does not benefit the anemia which develops in rice disease. The larger dosages actually accelerated the usual rate of blood destruction in both rice disease and in normal birds.

The ineffectiveness of iron administration on the anemia of rice disease extending over a 14 week period is explainable by the absence of a body iron deficit, in spite of the diminished iron intake. Preliminary histological studies have shown that the hemosiderin deposits in the livers of pigeons are most extensive in rice disease (in which the anemia is greatest), somewhat less after fasting, and least in normal birds. The body iron storage which parallels the development of anemia in fasting and in rice disease thus further indicates the importance of inanition as a causative factor in the development of rice disease.

With the exception of vitamin B, all known deficiencies of the polished rice when added to the diets of pigeons have been found ineffective in relieving or controlling the phenomena of rice disease. The vitamin B deficiency of polished rice therefore appears to be the primary if not the only causative factor in the development of this condition.

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THE INFLUENCE OF VITAMIN B ON THE INANITION, ANEMIA AND BACTERIEMIA OF RICE DISEASE IN PIGEONS

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In previous papers it has been shown that the phenomena of rice disease are not due to the absence of vitamins A, C, and D or of iron from the polished rice diet. On the other hand the blood changes may be largely corrected by means of cathartics, i.e., by adding magnesium sulphate or lactose to the rice diet, without influencing in any way the course of inanition. This beneficial action of the cathartics suggested, therefore, that the causative factor or factors in the development of rice disease might include toxemia or the bacteriemia which likewise develops, either as a result of the dietary vitamin B deficiency directly, or as a mechanical or chemical result of maldigestion and impaction of the rice.

The object of the present study was to determine the relative importance of the dietary vitamin B deficiency, the rice diet per se, and the bacteriemia and possibly toxemia as causative factors in the development of the rice anemia.

Methods. Three types of experiments were made, in each of which a sufficient number of pigeons were used to discount natural variability. In the first series, the maintenance value of various dosages of yeast extract was determined, when added daily to the diets at the beginning of the rice feeding. The weight and blood regenerating values of similar dosages of yeast extract were also observed when added to the rice diet after a 25 to 45 per cent body weight loss had developed. In the second series of experiments, a comparison was made between the changes which occur following the administration of polished rice or balanced synthetic (vitamin B free) diets to parallel groups of pigeons. The dietary corrective values of various dosages of yeast extract added to the synthetic diets were also noted.

In the third series of experiments, the possible relationship between bacteriemia or toxemia and the development of anemia was studied, by noting the influence of lactose on the weight and blood changes of rice disease.

The synthetic diet (Beard, 1926) consisted of purified (vitamin B free) casein 25 per cent, starch 38 per cent, crisco 21 per cent, cod liver oil 3

per cent, and the Osborne and Mendel (1919) salt mixture 7 per cent. This diet, like the rice, was fed with grit ad lib.

The observations taken were the same in all experiments and included the body weights, red cell volumes (Van Allen hematocrit), red cell counts, hemoglobin in grams per 100 ccm. of blood (Newcomer), and the degree of bacteriemia present. The bacterial counts of the blood were made by means of smears stained with Giemsa stain. The bacteria by this method were deeply stained. Their numerical ratio, in relation to the red cells, was determined by taking the median bacterial count of several fields of at least 500 red cells each and calculating the number of bacteria per cubic millimeter of blood. No attempt was made to identify the bacteria although both cocci and bacilli were observed.

RESULTS. *The influence of vitamin B on the course of rice anemia.* The influence of various dosages of Harris' yeast extract on the weight loss of pigeons receiving an exclusive polished rice diet is illustrated in figure 1. Each of the several curves, which will be referred to by letter, represent the median data from a series of experiments. The control groups A, B, and G (the latter taken from a similar study by Pilcher and Sollmann, 1927) received the following diets: polished rice, a basal, synthetic (vitamin B free) diet, and polished rice respectively. The deviation of the three independent experiments was negligible (maximal 3 per cent) at the end of the nine week feeding period and approximated a 47 per cent body weight loss. Group C received polished rice plus 150 mgm. of yeast extract daily. The body weight loss of this series during the first two weeks did not differ significantly from that of the rice control (A) group. The weight loss continued after the second week until approximately the sixth week but at a much slower rate than initially. After the sixth week, the body weight was maintained at 80 per cent normal during the succeeding five weeks of study.

Curves H, I and J represent similar experiments taken from Pilcher and Sollmann. The numerals 40, 80 and 120 represent the dosages of yeast extract in milligrams, administered daily per pigeon. The initial dissimilarity of curves C, H, I and J may be due to differences in the appetites of the birds for the rice. The terminal deviation of the weight curves of C (which received 150 mgm. of yeast extract daily) and of J (which received 120 mgm. of yeast extract) cannot be explained on such a basis, however, as no potency variation was noted in three separate samples of yeast extract with which group C was tested. The yeast dosage administered to this group therefore represents an 80 per cent maintenance value when added to the rice. The yeast extract administered to groups H, I and J on the other hand represent the hindering action of such dosages on the course of inanition rather than maintenance values since the weight losses were continuous.

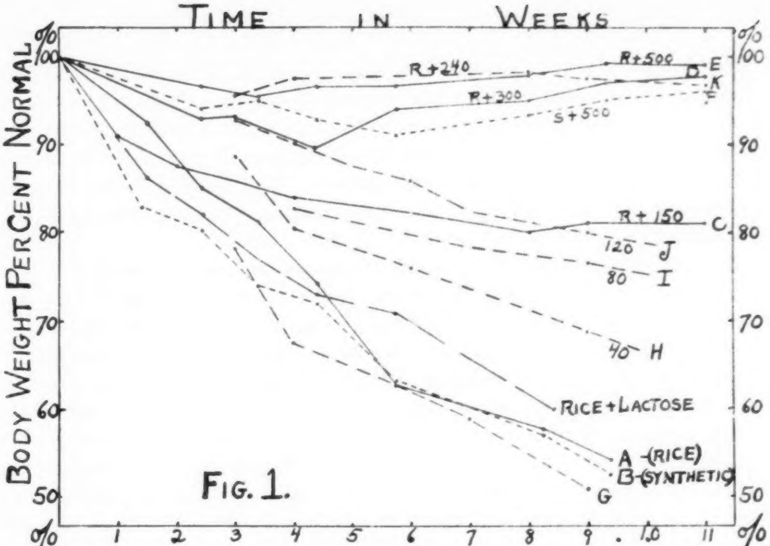

TIME IN WEEKS
BODY WEIGHT PER CENT NORMAL
R+240
R+500
R+300
S+500
R+150
120
80
40
RICE+LACTOSE
A-(RICE)
B-(SYNTHETIC)
E
D
K
F
C
J
I
H
G

FIG. 1.

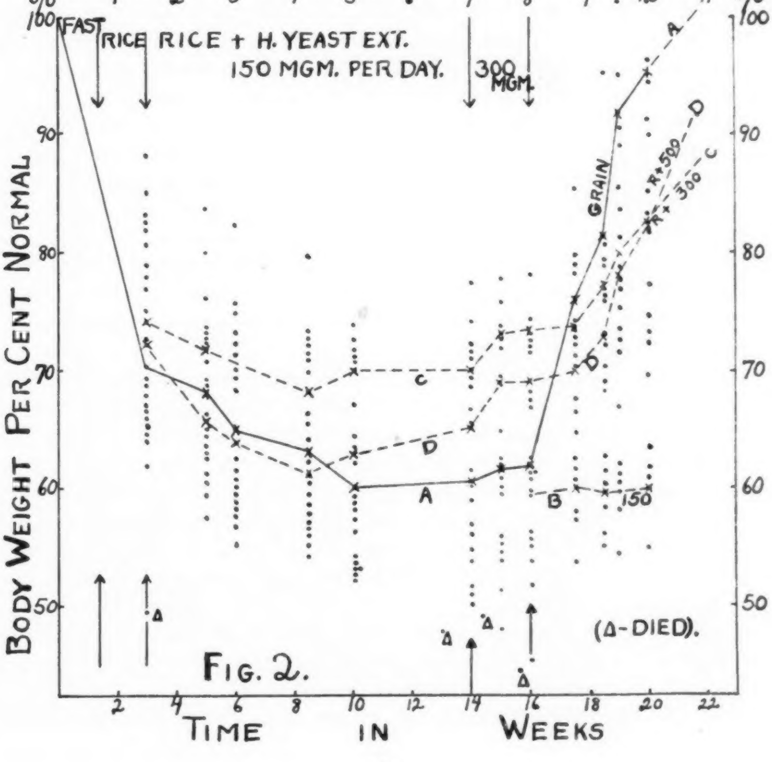

FAST
RICE
RICE + H. YEAST EXT.
150 MGM. PER DAY.
300 MGM.
BODY WEIGHT PER CENT NORMAL
GRAIN
R+500
R+300
A
B
C
D
150
(Δ-DIED).
TIME IN WEEKS

FIG. 2.

The addition of a sufficient daily dosage of vitamin B, i.e., at least 240 mgm. of Harris yeast extract per pigeon, to the rice diet forms a satisfactory basal diet as shown by curves D, E, and K. The dosages of yeast extract added daily to the rice diets of each pigeon of groups K, D, and E were 240, 300 and 500 mgm. respectively. The terminal body weight values for all three groups were quite similar and approximated the normal values.

The nutritional level of a group of pigeons receiving a polished rice diet plus an excess of vitamin B (500 mgm. yeast extract daily) figure 1 E, has been compared with that of a second group (F) of birds receiving a balanced, synthetic (vitamin B free) diet plus a similar dosage of vitamin B. The weight levels were maintained equally well in both groups over an eleven week period and no evidence of toxicity of the rice diet was noted.

The influence of vitamin B on the weight regeneration after the development of severe inanition. In order to cause a rapid 30 per cent body weight loss, a series of pigeons (fig. 2) of approximately equal weights, i.e., 325 grams, were fasted for 10 days and then fed a polished rice diet. From the 3rd to the 14th weeks each bird received an insufficient maintenance dosage, namely, 150 mgm. of yeast extract daily, and from the 14 to 16th weeks a just sufficient quantity, namely, 300 mgm. daily. The surviving pigeons were then divided into 4 groups to which the following diets were fed during the succeeding four weeks; group A, normal (whole mixed grain); group B, rice plus 150 mgm. of yeast extract; group C, rice plus 300 mgm. of yeast extract, and group D, rice plus 500 mgm. of yeast extract daily.

One of the most significant points illustrated by figure 2, in which each dot represents an individual body weight observation in terms of percentage of normal, is the wide deviation of the weight changes of different birds under a similar dietary régime, i.e., the divergence of the extremes from the median was about ± 10 per cent. Fourteen per cent of the entire group died by the 14th week.

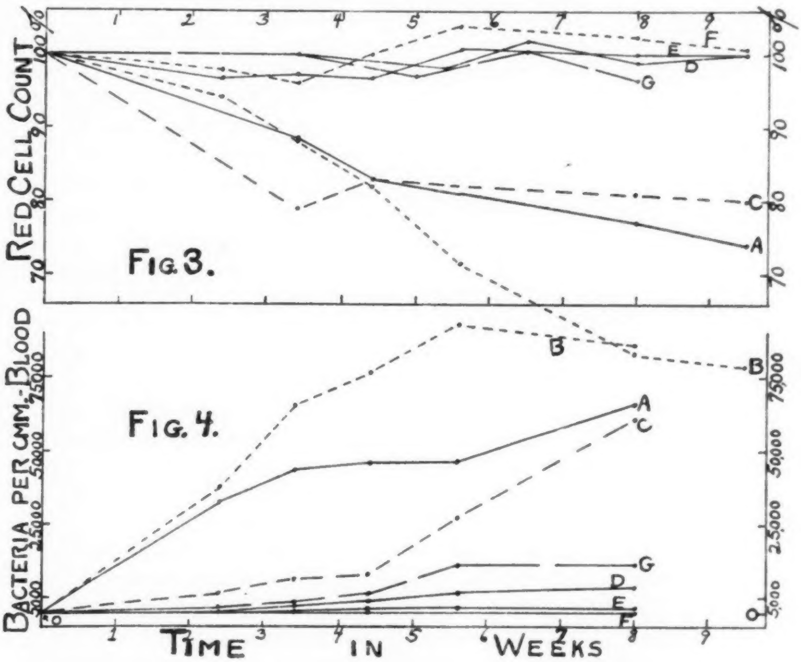
Increasing the daily yeast dosage of the entire series from 150 to 300 mgm. of the extract daily (the quantity adequate for normal maintenance) did not significantly alter the body weight levels during a two week period.

The substitution of whole grain for the rice plus yeast extract diet of group A, resulted in a progressive return of the body weight level toward normal. Recovery after this prolonged inanition period, however, was not complete until after 36 days, in contrast to the 7 to 12 day period necessary for recovery from a complete but briefer fast with a comparable weight loss.

Continuation of the rice diet with the yeast extract reduced to 150 mgm. (group B) barely maintained the weight level at 60 per cent normal, in contrast to the 80 per cent normal weight maintenance by similar dosages of extract when this was not preceded by a fast (fig. 1).

The median weight regeneration of group C (fig. 2) which received

300 mgm. of yeast extract, (the quantity adequate for the maintenance of a normal weight level when added to the rice diet of normal birds) showed a definite initial latent period, and in six weeks was less than half the weight which had been lost in the preceding treatment. A similar poor recovery was made by group D (fig. 2) which received an excess vitamin B dosage plus rice during the final four weeks of study. The improvement of the vitamin starved animals was definitely more rapid under the excess vitamin administration (D) than under the adequate vitamin dosage (C); although



supplementing a normal or rice diet with an excess of vitamin B has no effect on the weight of normal animals. It is therefore evident that the efficiency of vitamin B in controlling the body weight level, when used to supplement a polished rice diet, depends very largely on the degree of inanition present and the extent of the degenerative changes which have occurred. Consequently the direct proportion which has been shown to exist between the vitamin B dosage and the weight loss in rice disease, applies only if the animal has not already lost weight either by voluntary or forced fasting.

The influence of vitamin B on the red cell counts and volumes in rice disease. The changes which occurred over a nine week period, in the cell counts and volumes of two series of pigeons fed vitamin B free diets are illustrated by groups A and B, figure 3. Group A received polished rice while group B was fed the synthetic diet complete in all respects except vitamin B.

The rate of blood destruction in rice disease (fig. 3 A), as judged by the red cell count as a rule is always definitely slower and less marked than the corresponding weight loss. This is illustrated in figure 1 A, in which the weight level was 54 per cent normal after a nine week period, whereas the cell count at the same period was reduced only 26 per cent, i.e., 74 per cent normal.

The anemia which developed in group B, which was fed the synthetic diet, was definitely greater than that noted with polished rice over the same period of time. The terminal weight losses of groups A and B did not differ significantly, however. The terminal divergence of the cell counts of the two groups is probably due to the refusal of the birds of group B to eat the synthetic diet during the early part of the experiment although it may be dependent on other unrecognized factors.

The addition of a daily dose per pigeon of 150 mgm. of yeast extract to the rice diet of group C maintained the cell counts at a somewhat higher level than was observed with rice alone (A), but the differences of the cell counts of the two groups were smaller than those of the body weights. With a daily dosage of 300 mgm. of extract, the cell counts of group E which received a rice diet, were maintained within normal limits. The administration of an excess vitamin B dosage in addition to the rice diet (group C) was no more beneficial than the adequate maintenance dosage, i.e., the weights and cell counts were well within the normal range and did not differ noticeably from those of group E.

The synthetic vitamin B free diet when supplemented with a maintenance dosage of yeast extract (F) was capable of maintaining a normal cell count throughout the period of study. It would appear therefore that a diet of rice plus an adequate dosage of vitamin B is quite as efficient and no more toxic than a corresponding vitamin deficient but otherwise balanced synthetic basal ration similarly supplemented.

The cell volumes as well as the hemoglobin values of all groups corresponded so closely with those of the cell counts (in terms of percentage of normal) that their presentation seemed unnecessary.

Bacteriemia. In rice disease of pigeons, the depression of the red cell count per cubic millimeter practically parallels that of the body weight. The total corpuscle count, on the other hand, tends to decrease somewhat more than the body weight, while the ratio of blood weight to body weight increases, indicating the development of a relative hydremic plethora. The hemoglobin values are likewise decreased but as a rule to a somewhat

smaller degree than the red cells. The color index especially in severe anemia therefore becomes greater than unity. The development of both a relative and an absolute anemia as well as an increase in the color index might easily suggest the presence of some hemolytic agency in the genesis of the blood changes. Such a reaction, i.e., the increased blood destruction, might occur either as a direct or secondary effect following the altered intestinal flora or the bacterial invasion of the blood stream which has been shown to occur under similar dietary conditions in both birds (McCarrison, 1919) and mammals (Rose, 1928).

In the present study, the changes in the weight and blood which occur in rice disease have been correlated with the accompanying bacterial invasion in an attempt to determine: first, whether the anemia is primary or secondary to the bacteriemia, and secondly, whether the altered cell and bacterial counts may be varied independently, or occur simultaneously as secondary reactions to the dietary deficiency per se.

Parallel observations were made with several dietaries. These included polished rice, rice plus lactose (2 gms. per bird daily), synthetic (vitamin B free) purified diets, as well as additions of inadequate, adequate and excessive dosages of vitamin B to both the rice and synthetic diets.

Vitamin B free diets. The degree of bacteriemia which developed with B free diets was more extensive than with any other diet tested. The bacterial counts of groups A and D (fig. 4) which received polished rice and the B free synthetic diets respectively, did not differ significantly during the first three weeks of study. Terminally, however, the bacterial invasion was markedly greater with synthetic than with rice diets. It would appear therefore that the more important causative agent was the vitamin B deficiency rather than the diet per se. The extent of the blood invasion paralleled very closely (both chronologically and in degree), the anemia which developed.

Inadequate vitamin B diets. Supplementing a polished rice diet (C) with an inadequate B dosage (150 mgm. per pigeon daily) checked the bacterial invasion of the blood during the first five weeks of study. During this time, the maximal reduction of the red cell count had occurred. The bacteriemia, during the succeeding period, increased markedly and corresponded closely with that of the untreated series (A). The increased bacterial invasion of the blood which occurred after the fifth week of study is in contrast to the practically unchanged red cell count during the corresponding period.

The addition of such dosages of yeast extract to the rice diet resulted in a reduction of the weight and red cell counts which was approximately 50 per cent less than that which occurred in the untreated series A. It would appear therefore that the bacterial invasion was secondary to the dietary deficiency and was not directly concerned in the development of

the anemia, as an increase of 60 per cent in the bacterial count (after the fifth week) did not alter, during the succeeding four and a half weeks, the degree of anemia previously established.

Adequate and excess vitamin B diets. The addition of adequate or excess dosages of vitamin B (300 and 500 mgm. of yeast extract daily) to the rice diets of group E practically inhibited the bacteriemia; and no bacteria were observed in the blood of group F which was fed the synthetic diet plus the excess B dosage. The cell counts, volumes and hemoglobin values, as well as the weights of each of these three groups of birds remained within normal limits throughout the period of study.

Rice plus lactose. The addition of lactose to a rice diet, definitely limits or inhibits the usual anemia which occurs on a rice diet only (Barlow, 1927), although the course of inanition is unaffected. The invasion of the blood stream by intestinal bacteria is likewise markedly but not completely inhibited by the dietary addition of lactose (H). This beneficial action of the lactose on the rate of blood destruction and the development of bacteriemia, is not due to its negligible vitamin content since similar reactions are obtainable with other cathartics, i.e., magnesium sulphate and mineral oil. It must be attributed to the cathartic action, and to limitation and alteration of intestinal flora.

SUMMARY AND CONCLUSIONS

The several phenomena of rice disease, i.e., the loss of body weight, the development of anemia and bacteriemia, show a definite parallelism. These changes are not due to the rice diet as such, as they may be duplicated and even exaggerated by feeding a purified, vitamin B free, synthetic diet under comparable conditions.

The vitamin B requirements of pigeons receiving a polished rice diet vary markedly. With normal birds of similar body weights, voluntary starvation is the most significant factor in such variations; but pigeons with a history of a previous fast, or inanition as a result of vitamin B deficiency actually require higher dosages of vitamin B for either weight maintenance or regeneration, than do normal pigeons.

The parallelism of the bacteriemia and the development of the anemia, as evidenced by an increased rate of blood destruction, an altered blood volume, decreased hemoglobin level and an increased color index, suggests a possible causal relation either direct or indirect, between the invasion of the blood stream and the anemia. The parallelism however, although quite close in most respects, is incomplete and important divergences between the bacterial and red cell counts do exist. It is quite probable therefore that there are other factors concerned, at least in addition to the bacteriemia.

The addition of lactose to the polished rice diet shows that the bac-

teriemia as well as the anemia may be corrected by substances exclusive of vitamin B. The beneficial action of lactose must be attributed therefore to a diminution of the bacteriemia and possibly of the intestinal toxemia as well.

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THYROGLOBULIN VERSUS THYROXIN[1]

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The question of the hormone, or at least of the main hormone, of the thyroid gland seemed to be settled when Kendall announced his startling discovery of a comparatively simple crystalline principle which apparently possesses all the activity of the dried gland. While there is so far no evidence as to qualitative differences between the action of these two substances, some quantitative differences were found by Reid Hunt (1923); Cameron and Carmichael (1926); Boothby, Sandiford, Sandiford and Slosse (1925); and by Kendall and Simonsen (1928). Kendall (1928) assumes the existence in the thyroid of a slightly different unstable compound which he calls thyroxin.

While there is no evidence of the presence of free crystalline thyroxin or of the hypothetical active thyroxin in the thyroid or in its secretion, it has been established by the precipitin test that minute amounts of thyroglobulin are present (Hektoen and Schulhof, 1925); also that it is demonstrable as such in simple extracts from the thyroid gland and is present in the lymph and blood from the thyroid gland (Hektoen, Carlson, and Schulhof, 1927). The inference is plain that thyroglobulin is secreted by the thyroid.

If thyroxin as such—whether active or stable—is present in the thyroid secretion, it must have some different action than thyroglobulin or we would have the improbable example of a gland secreting two different substances, each with practically the same action. There is no reaction as sensitive as the precipitin reaction for thyroxin, and we know—and Kendall has shown—that thyroxin can be liberated only by a destructive procedure, while the presence of thyroglobulin may be demonstrated by simply diluting the fluid oozing from the cut gland with physiologic solution of sodium chloride. Other hormones, for instance epinephrine and insulin, may be obtained from the respective glands by mild procedures, although even those may be sufficient to hydrolyze a loose compound.

Whether Kendall's theory that crystalline thyroxin is an intermediary stage preceding the hypothetical active thyroxin, whether thyroxin is

[1] This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

changed by the process of isolation; whether thyroglobulin can account for all the action of the desiccated gland, are undecided questions. At present it seems worthwhile to learn all we can about the action of thyroglobulin, since it is the only active substance that is established as present in the thyroid secretion. We might expect that thyroxin or a compound

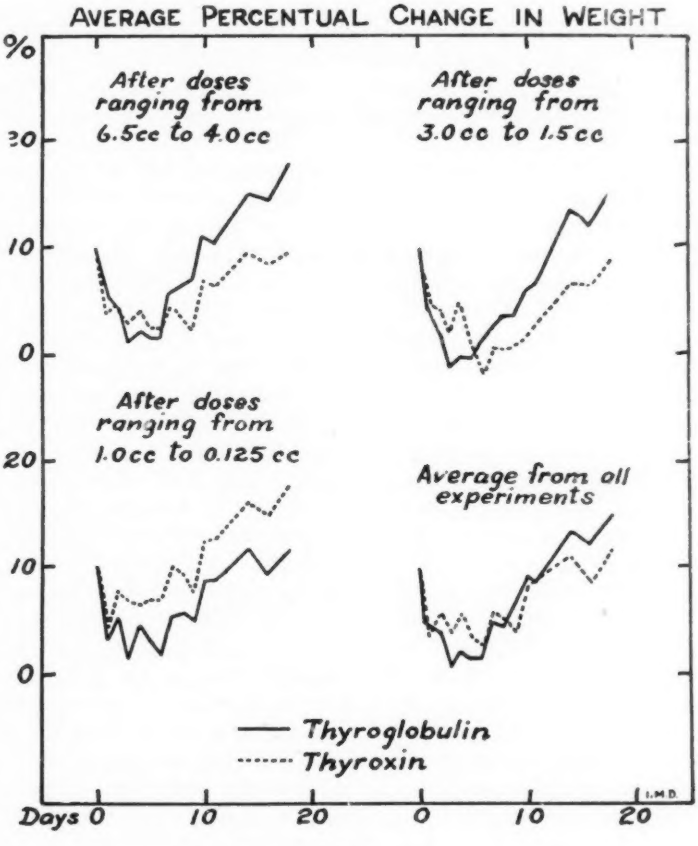

Fig. 1

similar to it, forming part of the large molecule of thyroglobulin, might be better adsorbed than a small molecule by the protein particles coming into contact with it. Furthermore, the high sulphur content of thyroglobulin might have a physiologic significance comparable with that of iodine, which is also electronegative.

This paper reports the results of certain experiments on rats. This animal has been successfully used in other experiments on the function of the thyroid gland. However, since the expected differences were only slight, the methods had to be tried and the probable error determined. The probable error was calculated as $u - \sqrt{\frac{\Sigma d^2}{n\text{-}1}}$, Σd^2 meaning the sum of the squares of the individual deviations from the average, n the number of observations (Brandt, 1926). The significance of the figure obtained in this manner is as follows: for instance, the probable error of a series of determinations of the basal metabolic rate on untreated animals is found to be $\pm$ 10 per cent of the average rate. This means that about three out of ten determinations of the rate made on untreated animals will show a larger deviation from the average than 10 per cent. About one out of twenty untreated animals may be expected to show a deviation exceeding the double probable error (in our example $\pm$ 20 per cent). The probability of deviations exceeding the higher multiples of the probable error is very small and consequently deviations of such order occurring in treated animals indicate that a real change has taken place. For instance, a deviation exceeding the threefold limits of probable error (in our example an increase or decrease in the metabolic rate amounting to 30 per cent) will occur in only one out of 333 untreated animals and the fourfold limit (40 per cent) will be exceeded in one animal out of 33,333.

First of all, Asher's phenomenon (Branovacky, 1926) of the increased sensitiveness of hyperthyroidized rats to a deficiency of oxygen was studied. From some of the data given by his collaborators I estimated the probable error of the original method at 10.9 per cent. I modified the technic by observing the metabolism and the behavior of rats in the apparatus described elsewhere (Schulhof, 1926). Instead of refilling the apparatus with oxygen during the test which took about three hours, water was used to replace the consumed oxygen. The probable error for the endpoint was 8.5 per cent. Unfortunately, the additional error due to an individually different reaction of the rats to the substance injected introduced a further difficulty in the quantitative estimation of the result and the method was abandoned. Similar considerations apply to the estimation of the basal metabolic rate. Nevertheless it seemed worthwhile to obtain further evidence of the qualitative action of thyroglobulin by testing its effect on the basal metabolic rate and on the weight of rats and to gain information necessary for further quantitative work on more suitable animals.

Beef and dog thyroglobulins were prepared by extraction of minced and slightly washed thyroids with physiologic solution of sodium chloride. The extract was centrifuged, filtered and precipitated by half saturation with ammonium sulphate, the precipitate redissolved and reprecipitated by a small amount of dilute acetic acid. This procedure was repeated.

The thyroglobulin solution was preserved with chloroform and filtered and reprecipitated with acetic acid before the experiment. The iodine content of the thyroglobulin was estimated according to the U. S. P. The final solution used was made to correspond in its iodine content to a solution of 10 mgm. of Squibb's crystalline thyroxin in 65 cc. of an alkalinized physiologic solution of sodium chloride.

Beef thyroglobulin was used in five rats tested for the change in the metabolic rate, while five other rats of corresponding weight ranging from 159 grams to 172 grams were injected with thyroxin: each animal received three injections on alternate days. Each dose was 1 cc., corresponding to 0.15 mgm. thyroxin.

The highest basal rates observed in the thyroglobulin animals were 25.6 per cent, 70.2 per cent, 56.5 per cent, 47.5 per cent and 58.9 per cent above the previous average basal rate taken from four to six preliminary estimations (not counting the first two tests made only for the purpose of training the animals). The rates in the thyroxin animals were 82.1 per cent, 42.7 per cent, 49.4 per cent, 61.2 per cent and 65.1 per cent above the previous rate. The average in the thyroglobulin animals was 51.7 per cent, in the thyroxin animals 60.1 per cent above the previous rates. The difference is well within the probable error of the method and allows only the conclusion that the action of preserved thyroglobulin and of thyroxin on the metabolic rate is practically the same in this experimental arrangement, if they are used in amounts equivalent in their iodine content.

Dog thyroglobulin was compared with thyroxin in the experiment on the action on the weight. Thirteen pairs of animals, each pair of equal sex and almost equal weight, were used. Two pairs of animals weighing more than the others received maximum doses (total of 6.5 cc., corresponding to 1 mgm. of thyroxin). Of the other pairs, the heaviest (266 grams) received the smallest dose (0.125 cc.). The dosage was gradually increased as the weight of the other pairs decreased, the lightest pair (122 grams) receiving the maximum dose of 6.5 cc. The dose was divided in two, each half being given on each of two successive days.

The results are given in the graph, the ordinates representing the average percentual change in weight per animal. The first impression is that thyroglobulin depresses the weight quicker and that the effect wears off faster than thyroxin. Only with lower doses was the recuperation faster with thyroxin. Yet the individual variations are too large to permit definite quantitative conclusions. For instance, while the average loss in weight in the thyroglobulin animals on the fourth day exceeded by 117 per cent the average loss observed in the thyroxin animals, the probable error in the former was 68.4 per cent, in the latter 85.2 per cent. Therefore even this large difference does not permit any safe conclusion. It is, however, at present sufficient to show that thyroglobulin has a marked

action on the basal metabolism and on the weight of rats. One grave objection, namely, that a foreign protein was used, may perhaps be overcome in experiments on dogs which also may be trained for the metabolism tests better than rats and from which blood samples may be taken at frequent intervals. In a few preliminary experiments on rabbits it seemed that certain changes of the blood may be a more rapid indicator of thyroid action than other tests. Much depends upon the constitution of the animal and the condition of the blood proteins may be more important for comparing of the experimental with the control animals than their weight. It seems probable that the condition (including the age) of the animal as well as the dosage might cause not only quantitative but also qualitative changes in the results.

A comprehensive study of many reactions in the same animal seems to me to be more important than separate studies of the single effects attributed to the thyroid substances. It might not be superfluous to emphasize that none of these actions are so specific that they could not be reproduced at least qualitatively with completely different compounds. If there is any specificity at all, I would look for it rather in the grouping of various actions peculiar to the thyroid than in single changes.

SUMMARY

The presence of thyroglobulin in the lymph and blood from the thyroid gland has been established, while there is no evidence for the actual secretion of free thyroxin. It is possible that the latter is split off from the thyroglobulin in the blood or organs, but thyroglobulin may have other active groups than thyroxin.

Injections of foreign thyroglobulins in rats have a marked action on the basal metabolic rate and on the weight. When injected in doses equivalent in their iodine content with thyroxin, the action of thyroglobulin is of the same order, at least within the quite large limits of the probable error of the methods used. Other methods which seem to be more promising are discussed.

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THE EFFECT OF ANTITHYROGLOBULIN SERUM ON THE PHYSIOLOGICAL ACTION OF THYROGLOBULIN[1]

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Various thyroglobulins on injection into rabbits induce the formation of precipitins which are specific in the sense that they react with thyroglobulin but not with the corresponding serum. By means of the precipitin reaction the presence of thyroglobulin has been demonstrated in the lymph and blood of the thyroid gland (Hektoen, Carlson and Schulhof, 1927). It seemed advisable to determine whether antithyroglobulin precipitin serum in any way counteracts the action of the corresponding thyroglobulin on the consumption of oxygen by rats.

The experiments were made on animals which had been repeatedly tested before. This was done partly in order to get them used to the procedure, partly in order to estimate the individual probable error, while general probable error with a larger number of rats and with the same apparatus (Schulhof, 1926) was determined at 10.75 per cent. Dog thyroglobulin which contained some serum globulin was used in a concentration of 1 per cent. The antiserum was prepared by injecting rabbits with a pure dog thyroglobulin. It precipitated in the ring test in contact with a thyroglobulin solution of 1:100,000. This serum was also injected as a control. Dog serum diluted 1:20 and an antiserum obtained by injecting dog serum into rabbits served as other controls.

The administration of thyroglobulin by mouth required too large doses and is difficult to control, if the rats are to get sufficient food. Therefore the doses which would raise the metabolism when injected, were determined in preliminary experiments. After that, three injections, each consisting of 0.5 cc. of the respective solution alone or of its mixture with another 0.5 cc. of the antiserum, were given at daily intervals and the basal metabolic rate again determined; then three or more injections of a double dose were given at intervals of two days and the metabolic rate determined at three day intervals. All injections were given subcutaneously.

The thyroglobulin was either injected alone or mixed immediately before the injection with an antithyroglobulin serum or with an antidog

[1] This work was aided by a grant from the Committee of Scientific Research of the American Medical Association.

serum. In the latter case precipitation took place, since the thyroglobulin contained some dog serum globulins, but this reaction was different from that obtained with antithyroglobulin serum and served only as a

TABLE 1

Results of experiments with antithyroglobulin

RAT NUMBER	AVERAGE BASAL METABOLIC RATE AND NUMBER OF DETERMINATIONS	PROBABLE ERROR AS PERCENTAGE OF METABOLIC RATE	WEIGHT	INJECTIONS	PERCENTUAL CHANGE IN RATE AFTER 3 INJECTIONS		MULTIPLE OF PROBABLE ERROR REPRESENTED BY THE CHANGE IN THE METABOLIC RATE IN THE PRECEDING COLUMN	
					0.5 cc. of each substance; total volume 1 cc. when two substances were injected	1.0 cc. of each; total volume 2 cc. when two substances were injected	Individual error	General error
1	2.02–10	8.1	137.5	Thyroglobulin	+20.0	+46.6	5.7	4.3
2	2.39–11	7.6	179	Thyroglobulin	+38.5	+52.3	6.3	4.5
3	2.26–10	10.5	188.5	Thyroglobulin + antithyroglobulin	+13.4	+33.2	3.1	3.1
4	2.01– 7	11.1	131.5	Thyroglobulin + antithyroglobulin	+21.9	+54.4	4.9	5.1
5	2.35– 8	8.7	157	Thyroglobulin + antithyroglobulin	+23.0	+48.5	5.5	4.5
6	2.42–12	5.8	177.5	Thyroglobulin + antidog serum	+3.2	+82.3	14.3	7.6
7	2.21–13	6.5	171	Thyroglobulin + antidog serum	+16.7	+89.6	13.8	8.3
8	2.07–13	12.7	125.5	Thyroglobulin + antidog serum	+11.6	+52.2	4.1	4.8
9	2.34–12	8.9	117	Antithyroglobulin	+41	−14.5	1.6	1.3
10	2.07– 3	4.1	131.5	Antithyroglobulin	−2.2			
11	2.25–15	11.8	139	Antidog serum		−9.3	0.8	0.9
12	2.35–12	8.4	195	Dog serum + antidog serum		+7.2	0.9	0.7
13	2.38– 8	11.4	165	Dog serum + antidog serum		−32.2	2.9	3.0
14	2.24–10	6.6	171	Dog serum + antidog serum		+14.3	2.2	1.3
15	1.92– 8	8.8	149	Dog serum + antidog serum		+12.0	1.4	1.1

closer control; a further control was secured by the injection of dog serum plus specific antiserum.

The metabolic rate was referred to a standard rat of 100 grams under

the assumption that the rate is a function of the square of the third root of the animals weight, as explained elsewhere. The results are given in table 1.

The average action of the smaller doses of thyroglobulin alone resulted in an increase of the rate by 29.3 per cent. The double doses increased it on the average of 49.5 per cent, which is 4.4 times greater than the probable error.

The action of the antithyroglobulin and of the antidog serum alone resulted in a slight depression of the metabolic rate, but this was too near the limits of simple probable error to be of value.

Although the average metabolic rate following the injections of the larger doses of dog serum with its antiserum was practically identical with the previous rate (within one-third of the probable error), it decreased in one of these animals by 32.2 per cent, which is three times the probable error and therefore apparently a real change. It seems evident that the action of a mixture of an antigen and antibody may be decisively influenced by the previous condition of the animal.

The results show clearly that the antithyroglobulin serum does not counteract the action of thyroglobulin on the metabolic rate; with the larger doses there is even a question whether it does not enhance it. However, even this action is hardly specific, since the addition of a mixture of another antigen and antibody (dog serum with its antiserum) to thyroglobulin increased its action more, but further experiments are required for final conclusion.

SUMMARY

The action of dog thyroglobulin on the basal oxygen consumption of rats is not counteracted by a simultaneous injection of antithyroglobulin serum.

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THE PACE MAKER OF THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS

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The ganglion on the dorsal surface of the heart of Limulus polyphemus originates the impulses which cause the contractions of the cardiac muscle (1). In large specimens the portion of the dorsal nerve cord which contains the ganglion cells may be 10 or even 12 cm. in length and one naturally wonders where in the extent of this long structure the impulse actually arises. Does it arise in a specific locus? Can it arise at different places? Does the whole ganglion function with each contraction, and if so what is the coördinating mechanism? These questions have been dealt with experimentally, treating the ganglion as if it were comparable and analogous to the pace maker of the vertebrate heart.

Section and excision: a. If a transverse section be made across the entire tubular heart at the level of the sixth segment, counted from the anterior end, the two pieces thus separated each contain about one half of the length of the ganglionated portion of the nerve cord. After the initial mechanical stimulation due to the operation the pieces settle down each to a perfect rhythm which measurement of tracings shows to be identical with the original rhythm, and each of the two pieces thus procured may be again cut into two sections and again each of the pieces will finally settle down to the original rhythm. The removal of the ganglion, i.e., the ganglionic cord and all outlying extensions which frequently are present, from any piece at once and forever stops all contractions of the muscle of that piece, proving the neurogenic origin of the beat as has been noted by Carlson. Two facts are noticeable: *a.* Although the rate of each piece remains the same as that of the original whole heart, the pieces no longer beat synchronously, but are out of phase; for example, while one piece is contracting another will be relaxing. The result is identical with that which Dr. Ida Hyde described for the movements of the gill plates of Limulus, which occur at a definite rate and in definite sequence, but if the ventral nerve cord is cut between the ganglia controlling each of the five gill plates the contractions of each gill plate continue at the same rate as before the operation but different ganglia are out of phase. These experiments indicate that the

inherent rhythm of the ganglion cells of all parts of the nerve cord of the Limulus heart is very approximately identical and that a coördinating mechanism exists such that all acting cells discharge their impulses with each contraction, i.e., that the ganglion normally acts as a coördinated whole in its discharge of impulses. *b.* The second noticeable result is the weakening of the height of contraction of any given segment as the pieces are decreased in size. That this is a result of decreasing the amount of ganglionic tissue connected with the contracting muscle is shown by another type of experiment in which records were taken from the second muscular segment. All ganglion cells are caudad to this segmental level, and the impulses which cause this second muscular segment to contract are con-

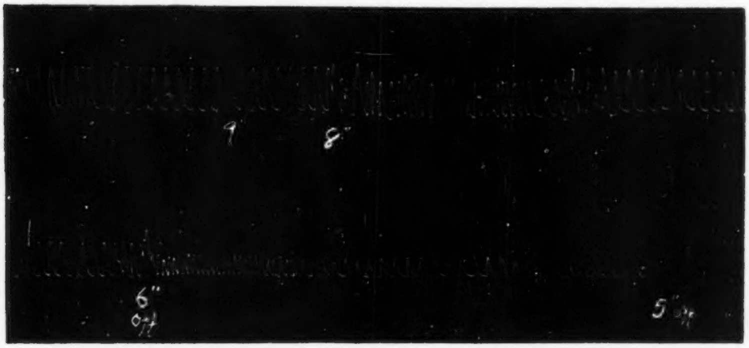

Fig. 1. The effects of progressive removal of the ganglion from Limulus heart: with progressive decrease in height of contraction. Return to normal rate in spite of the reduction in the amount of ganglionic tissue. The number represents the segment removed. Between the upper and lower tracing the ganglionic cord above segment 7 was removed. Note the temporary acceleration of rate due to cutting the ganglion at each level. Normal rate 20 beats per minute.

ducted forward from the ganglion by the median and two lateral nerves. If now one removes small bits of the ganglion piece-meal, working progressively from the posterior end forward, it is found that although the rate remains unchanged, except for the temporary acceleration due to cutting, the height of contraction of the anterior muscular segments decreases pari passu (fig. 1). Only one interpretation is admissible, viz., that all parts of the ganglion, although not necessarily all the ganglion cells, take part in the development of every impulse and that every part of the ganglion is connected with the cardiac musculature independently of the segmental arrangement. This conclusion is compatible with the anatomical description by Patten (2), i.e., with branching nerve fibers within the nerve cord and with their distribution by means of the median nerve and by lateral

branches to the lateral marginal nerve strands. Removal of pieces of the ganglion is tantamount to cutting off the nerve fibers from it to the muscle and Carlson (loc. cit.), Garrey (3) also, already have shown that cutting these nerve fibers to the muscle decreases the height of the contractions. The result is comparable to the cutting of the motor nerve fibers innervating a skeletal muscle, thus decreasing the number of contracting muscle fibers.

Having thus established the fact that under normal conditions the ganglion acts as a coördinated whole in the development of the impulses and that the inherent rhythmicity of all parts of the ganglion is practically identical, experiments were directed to the analysis of the conditions

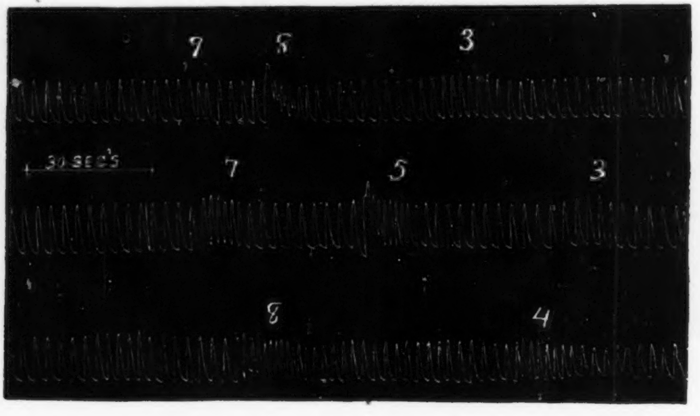

Fig. 2. Effects of applying a warm test tube to the ganglion at the segmental level indicated by the figures above the respective tracings. There is temporary accelerated coördinated rhythm, with return to normal rate after warming has ceased.

affecting individual parts of the ganglion. In this connection attention should be drawn to the fact that in the experiments described above cutting the nerve cord acts as a mechanical stimulus the response to which is an acceleration of the rate in which all segments participate coördinately and which may continue for several seconds or even minutes.

No matter at what segmental level the ganglion is cut, the sharply localized stimulus causes a reaction on the part of the ganglion as a whole. Evidently any small group of cells when stimulated can act as a pace maker and impress their rate upon other cells of the rhythm producing ganglion, as is likewise true of the rhythm producing foci of the vertebrate heart (Gaskell, 4).

Localized heating. Gaskell applied heat to the sinus of the cold blooded

heart and produced acceleration of rate; this was an effect solely upon the pace maker, for heating the ventricles caused no change in rate. In like manner MacWilliam (5) localized the pace maker of the mammalian heart by cooling and warming, and Garrey (6) has shown that it is possible by cooling one part of the turtle's sinus and warming another to shift the pace maker to the warmed region. Since warming the Limulus heart ganglion accelerates the rhythm (Carlson, loc. cit.; Garrey, 7), this method was resorted to in the production of localized changes which it was thought might localize the pace maker in definite and circumscribed parts of the ganglionic cord. In its crudest form the experiment was conducted as follows: a myogram was taken, any segmental level sufficing, although the second segmental anterior to the level of the ganglion cells was usually selected so that it was possible to work with the ganglion without disturbing the muscle record. The dry bottom of a small test tube containing heated

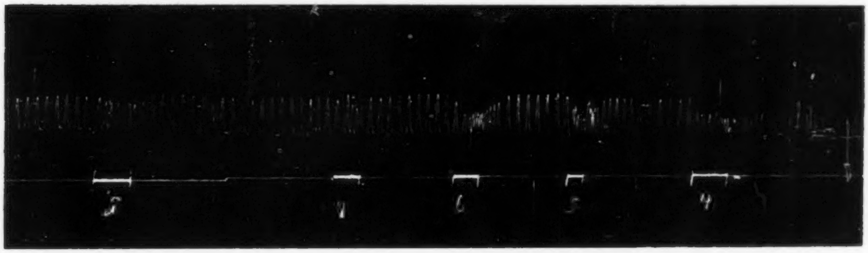

Fig. 3. The effects of heating one millimeter length of the ganglion of Limulus heart using an electro-thermal loop. Signals mark the duration of heating and the figures the segmental level.

sea water was applied at different levels to the ganglion, less than 0.5 cm. of the length of the ganglion was thus warmed, the remainder of the ganglion which in every case was from ten to twenty times the length heated, was kept at the original temperature. In every instance, and no matter at what level between the third and eighth segments the heat was applied, there was sharp acceleration of the rate while the localized high temperature was maintained (fig. 2). There being no ganglion cells anterior (cephalad) to the third segment no effects could be expected by heating this part of the nerve cord; the experiment thus serves as a means of determining the presence of ganglion cells and determining their anatomical location.

A smaller and more sharply circumscribed part of the ganglion was heated by the use of a tiny loop of fine resistance wire placed either under or against the nerve cord, at any level, and heated by closing a galvanic current. In this way a length not exceeding 2 mm. of the ganglionic cord was warmed and again in each instance there was an accelerated rhythm.

From these experiments we are justified in concluding that any small group of cells, no matter at what level in the ganglion they are situated, may become the pace maker and force the discharge of impulses from cells in all parts of the ganglion at the rate of those most active, i.e., of the heated cells (fig. 3).

Localized cooling. If a cold test tube (2°C.) is applied to a local spot on the ganglion no change in rate is observable. This result was expected as a deduction from the experiments described above. As long as any part of the ganglion remains at the original temperature which is above that of the new artificial experimental conditions then the part at the original higher temperature will determine the rate of the whole heart. Even if three-fourths or more of the ganglion is thus cooled there will be no change in rate. In fact when the whole ganglion is kept cold by covering it lightly with the bottom of a beaker or side of a test tube containing iced sea water and then heat applied to any localized spot on the ganglion, the rate of impulse formation is determined by the small region thus heated and is quite independent of the cold condition of the remainder of the ganglion, although exceptionally, owing to failure of normal conduction and therefore of coördination in the ganglion, the heated and cooled regions act independently and the muscle responds incoördinately and irregularly, the rapid rhythm being superimposed on the slow rhythm. These reactions in general are entirely comparable to the effects which one can induce in the cold blooded vertebrate heart in which the exact site of the pace maker may be determined or shifted at will by localized heating of the sinus venosus.

Localized stretching. Another factor which normally may come into play in determining cardiac rate in this animal is the degree of intracardiac tension. Carlson has demonstrated that when the intracardiac pressure is increased there is an increased rate, and ultimate incoördination if the pressure is excessive. We have noted in our experiments that when the anterior end of the animal was raised, changing the heart from the horizontal to the vertical position, the increased intracardiac pressure and consequent distention of the posterior segments of the heart completely altered the character of its contraction. Normally with the heart in the horizontal position the posterior segments beat approximately at the same time and as a unit (cf. Edwards, Pond, loc. cit.), but under the new experimental conditions in the vertical position the posterior segments beat first and the contractions progressed forward involving the anterior segments successively. This suggested the probability that there was a localization of the pace maker in the posterior end of the cord developed by stretching it. If a small hook or fine silk thread was passed under the ganglion, traction and the consequent slight stretching could be confined to a very localized region; this served to form a localized stimulus and to

establish a pace maker at the point stretched and as in other experiments, the coördinated impulses at the faster rate were developed by the ganglion, which in all these cases responded as a unit to the fastest rhythm of the experimental pace maker. Continued traction applied as above described, to the posterior end of the ganglion duplicated the effect of change to the vertical position and caused the posterior segments to beat before those

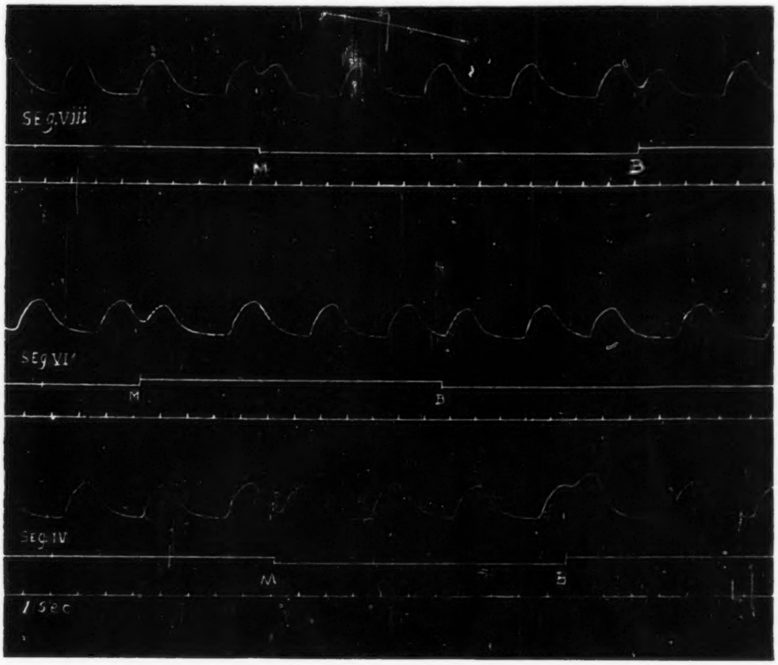

Fig. 4. Extra systoles produced by single induction shocks applied to the Limulus heart ganglion at any segmental level (indicated for each tracing). The extra systoles may be induced at any phase of contraction. There is no compensatory pause. The ganglion acts like the pace maker of the vertebrate heart.

anterior to it and there was a progressive involvement of segments from the posterior to the anterior end of the heart.

Localized electrical stimulation. A single induction shock applied at any point on the ganglion between the third and posterior segments (using electrodes with the two pints not over a millimeter apart) will produce an extra systole involving the entire musculature of the heart, thus demonstrating an evident coördinated involvement of cells throughout the entire extent of the ganglion. The heart thus responds with an extra systole

in exactly the same way the vertebrate heart does when the pace maker, i.e., the sinus venosus of the cold blooded heart, is stimulated, i.e., there is no compensatory pause and the normal rhythm is resumed after an interval only slightly exceeding the normal intersystolic interval (fig. 4). Analytic measurements of tracings showing the effects of extra systoles have been made by Prof. A. Samojloff (8), and his results appear elsewhere in this issue of THIS JOURNAL.

The effects of faradic stimulation are complicated by a number of factors which will not be considered in this communication beyond the general statement that if the stimulus be of selected intensity its application to any point in the course of the ganglion will accelerate the rhythmic discharge of the entire ganglion and force a rhythm determined by a localized pace maker.

It is inconceivable that all the multitude of ganglion cells throughout the course of the entire ganglion should always be in exactly the same physiological state at all times; therefore, the fact that they do discharge their impulses coördinately can only be interpreted as meaning that fortituous circumstances determine which cells will set the pace. It is conceivable, in fact it is highly probable, that this locus shifts from one part of the ganglion to another with inherent or local variations in physiological state. Carlson has noted that under certain conditions the posterior segments contracted before the others. That this is true when the pace maker is forced to the caudal end of the ganglion either by stimulation or by change to the vertical position has been noted above. Other reports indicate that the beat may begin in the middle segments (Carlson, 9). To settle this question Edwards (10) made accurate time measurements of the relative sequence of contractions in different segments of the Limulus heart and Pond (11) has also made a similar study. It is clear from results of these workers that the anterior segments beat later than the middle segments,—they are farther away from the rhythm producing ganglionated part of the cord and it takes time for the impulses to reach them. One may also conclude from their experiments that segments in the middle or posterior part of the heart which receive lateral branches from approximately common points on the ganglion beat together. Beyond these facts there is no evidence that there is an invariable sequence in contraction of the various segments. This may be interpreted as meaning that the pace maker has no fixed location, but may be located at different levels in different hearts, or may even shift in a given heart from one level to another where conditions, either natural, or experimentally induced, cause variations in the rhythmicity of a circumscribed group of cells.

SUMMARY

The ganglionated portion of the dorsal nerve cord on the heart of Limulus polyphemus, which is known to initiate the cardiac rhythm, is in every way comparable to the pace maker of the vertebrate heart.

The actual initiation of impulses may, however, be restricted to a very small group of nerve cells, and it is shown that it is possible by experimental methods to shift this pace maker group to any level of the ganglionated cord. In each such case a coördinating mechanism forces the rest of the ganglion to assume the rate of impulse formation impressed upon it by the most active cells. Normally this pace maker group has no fixed location and is determined by fortuitous circumstances.

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THE EXTRA SYSTOLIC IMPULSE OF THE GANGLION OF LIMULUS HEART

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The investigations of Carlson (1) on the heart of Limulus polyphemus have clearly shown that the muscle of this heart is under nerve control, that the automatic heart rhythm originates in the ganglion cells of the dorsal nerve cord of the heart and that excision of this ganglionic structure stops all contractions of the heart. We must, therefore, consider the ganglion as the cardiac "pace maker"—to use the modern term. Garrey (2) has extended the work on this cardiac ganglion. During the summer of 1929 Professor Garrey showed me the methods of preparing the heart for experimental work and demonstrated his unpublished work indicating clearly the "pace maker" characteristics of the ganglion. A test tube filled with warm water evoked an accelerated rhythm if applied to a localized spot anywhere on the ganglion. No other part of the heart when warmed gave this result. This experiment is analogous to the well known experiment of Gaskell on the respective effects of warming the sinus and ventricle of the frog's heart.

I spent some days in Woods Hole and occupied the limited time to conduct experiments to determine the duration of the interval between an extra systole produced by electrical stimulation of the ganglion and the next normal spontaneous beat of the heart. It was my objective to determine in this way whether the ganglion behaved like the pace maker of the vertebrate heart with only a slight lengthening of the post extra systolic interval, or whether there ensued a compensatory pause like that which results upon stimulating other parts of the vertebrate heart—the ventricle, for example.

In preparing the experiment the dorsal nerve cord was cut at about the level of the third segment from the anterior end, thus separating the anterior nonganglionated fibers (*a*-fig. 1) from the posterior ganglionic portion (*G*-fig. 1). The anterior fibers were dissected away for a short distance to avoid stimulating them. The anterior end of the ganglionic portion was placed upon electrodes (*E*-fig. 1) connected with an induction coil and single induction shocks used to produce extra systoles. The muscular contractions were recorded by attaching the lateral margin of the heart by a thread to a lever writing on the smoked paper on a kymographion.

In figure 2 we see the curves of a contracting Limulus heart with a time tracing recording seconds. At two places (*A* and *B*) there are extra systoles and it is easy to measure the duration of the normal intersystolic intervals and of the interval between the beginning of an extra systole and the beginning of the next normal systole. These measurements follow in table 1, likewise those of another curve.

The measurements show that the post extra systolic interval exceeds the normal period by only a slight time difference and we can say that the ganglion behaves in this regard like the pace maker of the vertebrate heart

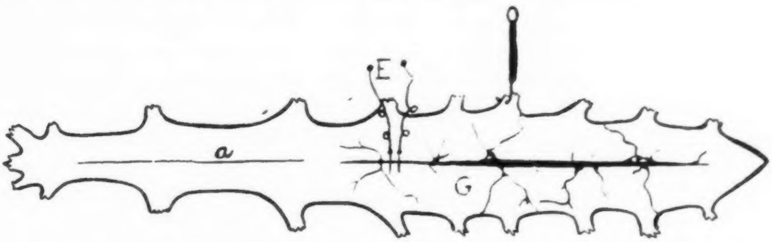

Fig. 1. Diagram of Limulus heart (from photograph) showing the segmental attachments and method of preparation for electrical stimulation of the dorsal ganglion. *a*, anterior median nerve cord; *G*, dorsal ganglion; *E*, electrodes for stimulation; a hook through the lateral attachment between the 5th and 6th segments is attached to a recording lever.

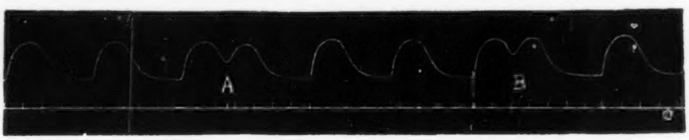

Fig. 2. Record of the rhythmic contractions of a Limulus heart. Extra systoles caused by simple induction shocks applied to the ganglion are shown at *A* and *B*. Time in seconds.

for the same relation is often found for extra systoles produced by stimulating the sinus of the frog.

We know from experiments on the vertebrate heart that the extra systole of the ventricle gives one post-extra systolic compensatory pause. This raises the question whether it is possible to produce a compensatory pause by stimulating the heart muscle of Limulus heart. Experiment shows that this cannot be done. Excitation of Limulus heart muscle evokes a purely local contraction limited to the area stimulated. When the muscle is stimulated by a single induction shock its contraction (extra systole) generally cannot be recognized on the tracing. A short faradic

TABLE 1

DURATION OF NORMAL INTER-SYSTOLIC INTERVAL	DURATION OF THE POST EXTRA-SYSTOLIC INTERVAL
seconds	*seconds*
4.5	
4.5	4.5
4.4	
4.5	4.7
4.5	
Average.........4.48	4.6
4.5	4.7
4.5	4.7
4.5	
4.5	4.8
4.5	
4.6	
4.6	4.9
	4.7
4.7	4.9
Average.........4.55	4.75

Fig. 3. Brief faradization of the muscle shown at two places. The arrows indicate the beginning of normal neurogenic contractions superposed on the muscle tetanus without any interruption of normal rhythm. Time tracing marks seconds.

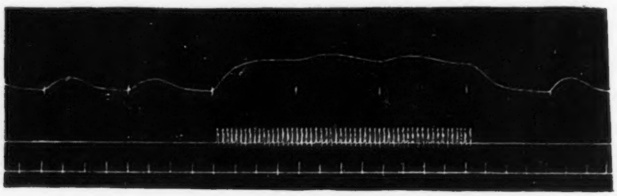

Fig. 4. Weak faradic stimulation of the muscle produces a mild grade tetanus with the normal neurogenic contractions superimposed upon it. Time in seconds.

excitation of the muscle calls forth a short tetanus which can be well distinguished on the curve, but which exerts no influence on the rhythm of the whole heart. In figure 3 a short tetanus of the muscle begins a little before a normal systole and for this reason appears like an extra systole, but measurement shows that the time from the preceding normal systole to the excitation contraction plus the succeeding interval is exactly equal to that of two normal systolic periods; we see here the same time relation as in cases of compensatory pause in the vertebrate heart after a single ventricular extra systole. It is, however, easy to understand that our extra systole is a combination of a localized short muscular tetanus with the systole of a normally innervated part of the heart; in fact, careful examination reveals the exact instant when the impulse from the ganglion stimulates the muscle noted on the tracing by the small arrows. The normal systolic time interval further proves its normal ganglionic origin.

If the tetanus is of longer duration as in figure 4, the whole curve is elevated and the normal rhythmic contractions are superposed on the curve and can be seen to proceed without interruption unless the tetanizing excitation is very strong when other disturbances are noted.

CONCLUSION

These experiments demonstrate conclusively that in its reaction to artificially induced extra systoles, the ganglion of the Limulus heart shows all the features characteristic of the pace maker of the vertebrate heart. We must come to the conclusion that the mechanism of the automatism is the same whether it develops in the heart muscle fiber of the vertebrate heart or in the nerve cells of the ganglion of Limulus heart.

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THE CARBON DIOXIDE DISSOCIATION CURVE OF FROG HEART MUSCLE

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It has been found by several investigators (Katz and Long, 1925; Arning, 1927; Gemmill, 1928; Boyland, 1928) that cardiac muscle has a lower resting lactic acid content and a lower lactic acid maximum than skeletal muscle of the same animal. This has been taken to mean a lesser buffering capacity of cardiac tissue. Furusawa and Kerridge (1927), using a glass electrode, determined the dissociation constants for the total buffers of heart and skeletal muscle by titration with lactic acid. They found a lower value for cardiac than for skeletal muscle.

It was thought possible to confirm these findings in a totally different way by measuring the carbon dioxide dissociation curve of heart muscle. Fenn (1928) in his paper on the carbon dioxide dissociation curve of frog muscle and nerve pointed out the significance of the tissue dissociation curve in this connection. By tipping acid on hearts which have been equilibrated with different tensions of carbon dioxide, the carbon dioxide combined in the heart at each tension was measured volumetrically. The dissociation curve for heart muscle so obtained is compared with the results of Fenn for skeletal muscle.

Method. The apparatus used was the differential volumeter (Fenn, 1927) fitted with Pyrex chambers, with outlet tube and stopcock to permit equilibrating with gas mixtures, and having a shelf on which the piece of tissue rested. Five-tenths cubic centimeter of 0.4 M hydrochloric acid was placed in the bottom of the chamber instead of the alkali usual in oxygen consumption measurements. The ventricles were removed from adult Rana pipiens, and were washed free of blood with unbuffered Ringer's solution. They were then placed upon the shelf in the bottles already containing the 0.5 cc. of acid, and the volumeter was immersed in a thermostatically controlled water bath. Oxygen-carbon dioxide mixtures were made up in 20 liter bottles. The gas was then passed through the bottle containing the heart. Samples of the gas were drawn into sampling tubes and analysed in a Henderson-Haldane gas analyzer.

Volume readings were taken until a constant rate was attained. Even

if the R.Q. was exactly 1.0 before tipping the acid, a slight diminution in volume would still be expected under the conditions of these experiments because of the retention of some of the carbon dioxide by the tissue. If the R.Q. is less than 1.0, the diminution of volume is still greater. In accordance with this expectation, fourteen of the nineteen experiments here reported show an initial decrease of volume as indicated by a movement of the index drop towards the heart chamber. The apparent R.Q. greater than 1.0 in the other five cases may possibly be due to incomplete vapor pressure equilibrium in the volumeter.

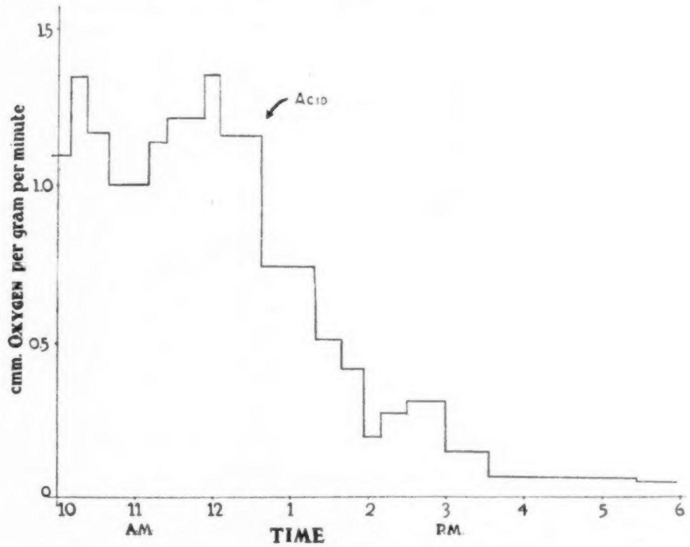

Fig. 1. Graph showing the effect of acid upon the oxygen consumption of frog cardiac muscle. The ordinates represent cubic millimetres of oxygen consumed per gram of tissue per minute. The abscissa represents time in hours. At 12:30, as indicated by the arrow, acid was spilled upon the tissue.

The interpretation of the movements of the drop after the tipping of the acid is complicated by the fact that the drop seldom comes to a complete rest. Usually it moves slowly away from the heart chamber for a period varying from one-half to two hours as the carbon dioxide is evolved and then reverses and moves slowly back again. This reversal suggests that in spite of the acid some gas exchange still persists within the heart which tends to diminish the amount of movement observed. This suggestion was confirmed by the experiment of figure 1 in which it was arranged to tip acid onto a heart in the presence of sodium hydroxide within the

chamber so that movements of the drop would indicate oxygen consumption. The result shows after the spilling of the acid an immediate depression of the oxygen consumption to about 50 per cent followed by a further gradual diminution to a rate less than 10 per cent of the original, and persisting for several hours. The graph shows that this final rate persists throughout most of the period of evolution of carbon dioxide after acidification and justifies the use of the final rate of movement of the index drop in preference to its initial rate as a base line from which to calculate the

TABLE 1

EXPERIMENT NUMBER	WEIGHT OF VENTRICLE	CO_2 TENSION	CO_2 COMBINED
	mgm.	*mm. Hg*	*vol. per cent*
1	104	0*	5.6
2	104	0	6.2
20	93	0	7.7
21	82	0	12.6
22	85	18.0	11.9
23	77	18.0	9.1
18	114	32.6	11.2
27	85	39.6	7.4
5	128	40.2	12.1
26	85	42.4	8.6
17	133	86.7	13.6
16	135	124.5	16.1
14	66	131.2	11.0
11	121	152.3	19.8
15	101	169.2	16.4
13	95	175.7	19.0
24	59	171.3	17.3
12	124	190.0	16.1
9	75	194.0	20.0

* The tensions as given refer to the tensions of CO_2 in the mixture with which the volumeters were flushed. The actual tensions within the tissue were actually higher, as has been explained in the text. It is understood that there never was actually zero CO_2 tension in any of the experiments.

volume of carbon dioxide evolved. This correction was applied to all the determinations reported in this paper. Since it is a minimum correction, all the results are probably very slightly too small. This error is inconsiderable since the total correction averages only 6 per cent of the value given, and the largest individual correction was 1.8 volumes per cent.

Results. The results from the nineteen experiments are given in table 1. All the values for the carbon dioxide displaced by acid from combination have been expressed in terms of volumes per cent. They are plotted against carbon dioxide tensions as abscissae in the middle curve of

figure 2. The exact shape of the curve could not be accurately determined; the dots show the scatter of the experimental values. The dotted line shows the probable position of the "true" curve after correcting for internal carbon dioxide tension. On the same graph is drawn the lowest of the two dissociation curves given by Fenn (1928) for frog skeletal muscle.

The heart curve is distinctly lower, and is a much flatter curve. Both these findings indicate a lesser ability on the part of cardiac muscle to buffer a change in pH. The pH lines, which have been drawn in the figures, have been calculated from the Henderson-Hasselbalch equation,

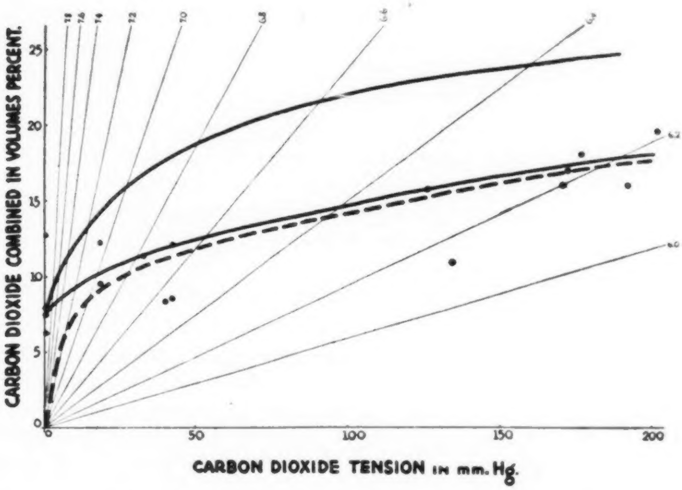

Fig. 2. The carbon dioxide dissociation curves of skeletal and cardiac muscle in the frog. The upper curve is Fenn's curve for skeletal muscle. The middle curve is for heart, drawn through the experimental values. The lower curve is corrected as explained in the text.

using a value of 6.17 for pK_1, as given by Cullen, Keeler and Robinson (1925) for human blood, and used by Fenn and by Stella.

If one calculates the buffer value β, as defined by Van Slyke (1922), one arrives at a figure of 0.0045. This is to be compared with Fenn's value of 0.01 for frog's skeletal muscle and 0.02 for frog's blood (calculated by Fenn from the data of Wastl and Seliskar (1925)).

Discussion. Even when the heart is in pure oxygen, the tension inside the tissue is not zero because of delay in the diffusion of carbon dioxide. Approximation of this increased tension is difficult to make due to the heart's irregular geometric form. The ventricle walls were between 1.5 and 2.0 mm. in thickness, but due to the spongy construction, its functional

diffusing thickness is probably less. Assuming that the heart is a flat sheet of tissue, 2 mm. thick and freely exposed for diffusion on both sides, it may be calculated, as Stella did (1929) for skeletal muscle, from the formula given by Hill (1928, p. 51) for the concentration of lactic acid in skeletal muscle in oxygen-free Ringer's solution, that there are 1.1 volumes per cent of "diffusible, physically dissolved carbon dioxide" within the muscle in oxygen in the steady state. This corresponds to an average tension of 10 mm. Hg. The broken line in figure 2 is drawn so that it would pass through the experimental points if they were all displaced 10 mm. Hg to the right, thus taking account of this error.

Stella (1929) has suggested that this error can be obviated by carrying out the determination in mixtures of carbon dioxide and nitrogen as he did for skeletal muscle. It is doubtful, however, if much is gained by this modification of the procedure; for measurements of the anaerobic carbon dioxide evolution from heart show that during the first hour of anaerobiosis, carbon dioxide is still coming off at a rate which is greater than half the aerobic rate. Possibly Stella's procedure is better adapted to the better buffered skeletal muscle where, according to preliminary measurements made in this laboratory, the rate of anaerobic carbon dioxide formation is less than in heart or in nerve.

But although the use of nitrogen in place of oxygen may obviate to some extent the difficulty just mentioned, it involves another equally serious error, namely, that due to anaerobic lactic acid formation. Stella has estimated the amount of this error as 8 per cent of the combined carbon dioxide at every tension. It seems instructive to estimate this somewhat more accurately. This has been done by two methods; a graphical method, and from the titration data of Furusawa and Kerridge (1922), both of which lead to identical results.

It is evident that if the lactic acid formed in nitrogen be so added to the tissue that the pH remains constant, the entire amount of bases necessary for its neutralization would have to come from bicarbonate. In that case, the carbon dioxide tension would have to fall to the value determined by the remaining combined carbon dioxide at the pH in question. Therefore, knowing only the tension and combined carbon dioxide measured in nitrogen and the amount of lactic acid which has been formed in the anaerobic period, it is possible to arrive at a point on the curve representing the combined carbon dioxide at the beginning of anaerobiosis. It is merely necessary to erect a vertical (*ab*, fig. 3) from the point, *a*, equal in length to the carbon dioxide equivalent of the added lactic acid, and at the end of the verticle, draw a horizontal line (*bd*, fig. 3). The point, *d*, at which this line intersects the pH diagonal through the initial point, *a*, is a point on the desired dissociation curve.

In figure 3, the construction just outlined has been made on Stella's

curve. The solid line is drawn through the values given in Stella's table 4. The dotted line drawn through the points indicated by triangles has been determined, assuming a lactic acid production of 0.2 mgm./gm. of muscle, which is equivalent to 5 volumes per cent of carbon dioxide. By taking the values from the curves for the carbon dioxide displaced at each tension, the proportions of the lactic acid buffered by bicarbonate and other buffering substances can be arrived at. These are shown in table 2. It

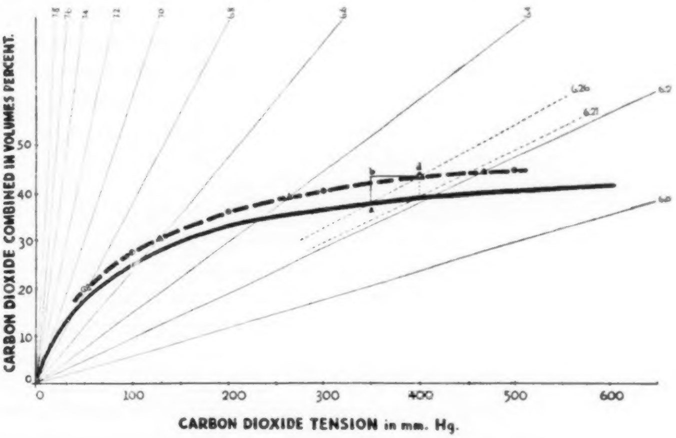

Fig. 3. The solid curve is drawn through the values given by Stella for the carbon dioxide dissociation curve of frog skeletal muscle. The broken curve has been obtained from it in the ways explained in the text.

TABLE 2

	CO_2 TENSION				
	100 mm. Hg	200 mm. Hg	300 mm. Hg	400 mm. Hg	500 mm. Hg
Per cent of CO_2 buffered by bicarbonate......	50	70	80	90	95

can be seen from these data that the part of the lactic acid buffered by bicarbonate increases with increasing carbon dioxide tension. At tensions prevailing in the intact animal, lactic acid must be largely buffered at constant CO_2 tension by substances other than bicarbonate.

The second method uses the data of Furusawa and Kerridge. Between pH 6.0 and pH 7.0, Furusawa and Kerridge (1927) found that the buffer curve for mammalian skeletal muscle is a straight line. The addition of 4.1 mgm. of lactic acid per gram of muscle causes a unit change in pH. Taking Meyerhof's (1920) figure, as used by Stella, of 0.2 mgm. per gram

per hour as the rate of anaerobic lactic acid formation, and assuming that this rate is independent of the carbon dioxide tension with which the muscle is equilibrated, the lactic acid produced in one hour will cause a decrease of 0.05 in the pH of the muscle. By applying this change to the Henderson-Hasselbalch equation at every CO_2 tension, the change in the carbon dioxide combining power at each tension, due to the formation of lactic acid, may be calculated. This has been done at various tensions for Stella's curve. The points within a circle in figure 3 were derived in this manner. It can be seen that they fall exactly upon the curve arrived at by the graphic method. This is surprising, for the application of Furusawa and Kerridge's data necessitates the assumption that the slope of their buffer curve in the range used is independent of the carbon dioxide tension.

In this approximation of the "true" curve for "fresh, resting muscle" from Stella's data no account has been taken of the increased tension of carbon dioxide due to the same lactic acid formation. As Stella has shown, the tension within the muscle, at highest carbon dioxide tensions, would be about 4 mm. higher than the external atmosphere. At lower tensions, due to the better buffers, this would be less.

The dissociation curve would further indicate that a difference between skeletal and cardiac muscle is to be explained by a lesser buffering capacity.

SUMMARY

1. The carbon dioxide dissociation curve of frog heart muscle has been determined and found to be about one-third lower than that determined for frog skeletal muscle by Fenn.

2. The buffer value (β of Van Slyke) is about one-half as great for heart muscle as for skeletal muscle of frog.

I want to express my thanks to Dr. W. O. Fenn whose kind help was always at my disposal, and at whose suggestion this problem was undertaken.

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STUDIES ON VENTRICULAR FIBRILLATION PRODUCED BY ELECTRIC SHOCK

III. The Action of Antagonistic Salts[1]

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In a first communication (Wiggers *et al.*, 1930) facts were presented to show *a*, that ventricular fibrillation produced by faradic stimulation of the dog's ventricle runs an average course in 24 minutes, after which revival of the circulation and central nervous system is rarely possible; *b*, that injections of KCl solution, not less than 50 mgm. per kilo into the two ventricles, is capable of abrogating fibrillation promptly, the ventricles coming to complete rest in diastole; and *c*, that subsequent injection of corresponding doses of $CaCl_2$, combined with massage, frequently restores a normal beat and normal blood pressures, after which normal reflexes can be elicited from the spinal cord, medulla and mid-brain.

In a second communication (Wiggers, Bell and Paine, 1930) the natural course of fibrillation and its modification by intraventricular injections of KCl solutions were analyzed through the use of cinematographic and electrocardiographic methods. Such studies demonstrated that KCl arrests ventricular fibrillation by hastening the natural series of events and without the induction of new phenomena. It was also demonstrated that revival after subsequent use of $CaCl_2$ and massage is inaugurated by a rhythmical series of idioventricular beats which are, however, superseded by a supraventricular rhythm. It remains to discuss various details of the action of these antagonistic ions which have hitherto been mentioned only casually or have not been reported at all.

Potassium as a cause of fibrillation. The depressant action of potassium ions on the functions of rhythmicity, contractility and excitability have been repeatedly established both on perfused and intact hearts (cf. Sollmann, 1926). Our interest centered largely upon reports of numerous investigations (Traube, 1864; Guttmann, 1866; Boehm, 1878; Aubert and Dehn, 1874; Mathison, 1911; Hering, 1915; and McWilliam, 1918, among

[1] Published by The Physiology Committee on Electric Shock.

them) that intravenous injections of doses less than those suggested to abolish fibrillation actually cause or predispose normally beating hearts to ventricular fibrillation. Thus, while we suggested minimal doses of 50 mgm. per kilo and Hooker (1928, 1929) advised approximately 125 mgm. per kilo for arresting fibrillation, Aubert and Dehn (1874) believed they had demonstrated doses larger than 8 mgm. per kilo to be lethal for dogs and rabbits. Mathison (1911) also produced death in cats by administering the equivalent of 55 mgm. as a 1.1 per cent solution. Assuming an average weight of 2 kilos for a cat, the lethal dose was decidedly less than that employed either by us or by Hooker.

It was important not only to confirm these observations but if possible to find an explanation for this paradoxical action—and particularly so, as its practical employment to arrest fibrillation is contingent upon the *modus operandi.* To do this, it was essential that we compare the fibrillation by potassium produced with that induced by faradic stimulation.

In various experiments KCl in 1 per cent and 5 per cent solutions and in different doses was injected intravenously, i.e., into a saphenous, femoral or jugular vein, or directly into the right auricle by a catheter. The rate of injection was also varied. Optical pressure curves from the ventricles and aorta and electrocardiograms by lead II were simultaneously recorded.

The different effects obtained are worthy of rather detailed report. Smaller doses, 8 to 10 mgm. per kilo, could be repeatedly injected as a 1 per cent solution with impunity. Occasionally the blood pressure rose or fell moderately, but a careful analysis of records failed to reveal any essential changes in the pressure curves or electrocardiograms. Segment A of figure 1 shows the pressure curves immediately after the injection of the last of such cumulative doses, 38 mgm. per kilo having been administered up to the time these records were taken. No deviations from the normal could be detected by the most careful measurement. Much larger doses, i.e., up to 210 mgm. per kilo, were also injected without induction of serious disturbances. Such results, however, do not demonstrate the harmless character of equivalent doses of KCl when introduced directly into the ventricular cavities of fibrillating hearts. Since the circulation is arrested, the potassium salts introduced directly into the ventricular cavities were diluted only by the residual blood in the ventricles instead of as normally by the total blood in the circulation.

Our interest obviously lay in the effects of more concentrated doses. As others have pointed out, the toxicity of potassium salts in naturally beating hearts is determined not only by the actual dose but by the concentration which reaches the heart muscle. Thus, Mathison (1911) found larger doses could be tolerated by intra-arterial than by intravenous injections, chiefly owing to differences in dilution. D'Halluin (1926) claims to have introduced even up to 300 to 420 mgm. KCl per kilo by very

slow injections (4 mgm. per kilo per min.), while 200 mgm. per kilo injected more rapidly proved disastrous.

We found that doses ranging from 30 to 100 mgm. introduced rapidly into the right auricle or jugular vein usually produced abnormalities of the

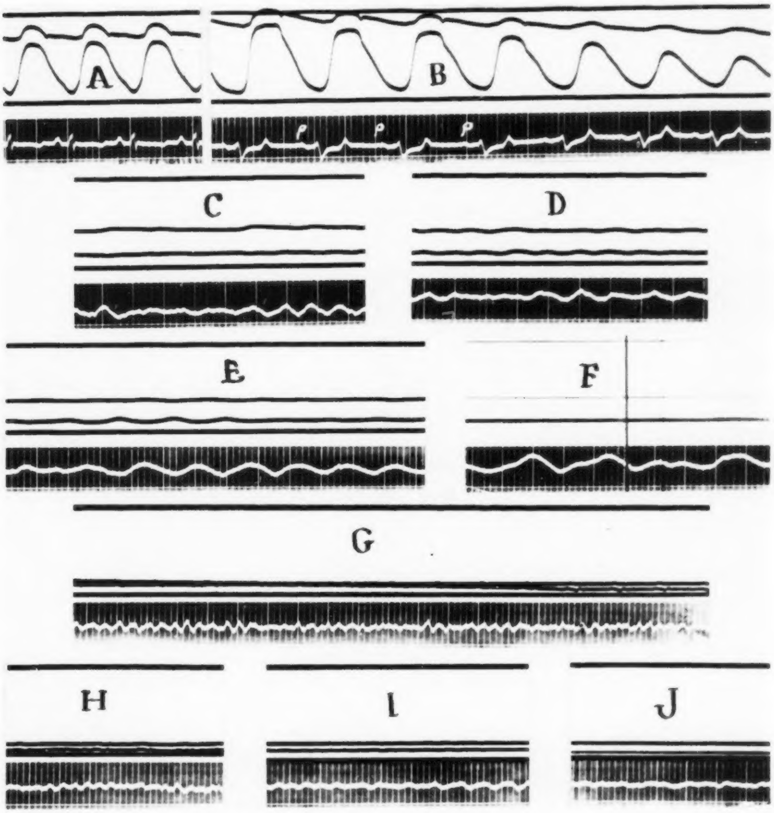

Fig. 1. Aortic and intraventricular pressure curves and electrocardiograms (lead II), illustrating development of so-called fibrillation after KCl and a comparison with true fibrillation. A, control; B-F, progressive changes due to 63 mgm. KCl per kilo via jugular vein; (expt. O-81); G-J, fibrillation due to faradic stimulation (expt. O-79), used to contrast with E-F. Time 0.04 and 0.2 second. Description in test.

pressure and electrical curves and led rapidly to the state designated as fibrillation. Thus, in the experiment illustrated by the records of figure 1, B *et seq.* 25 mgm. per kilo, in addition to the previous use of 38 mgm. in

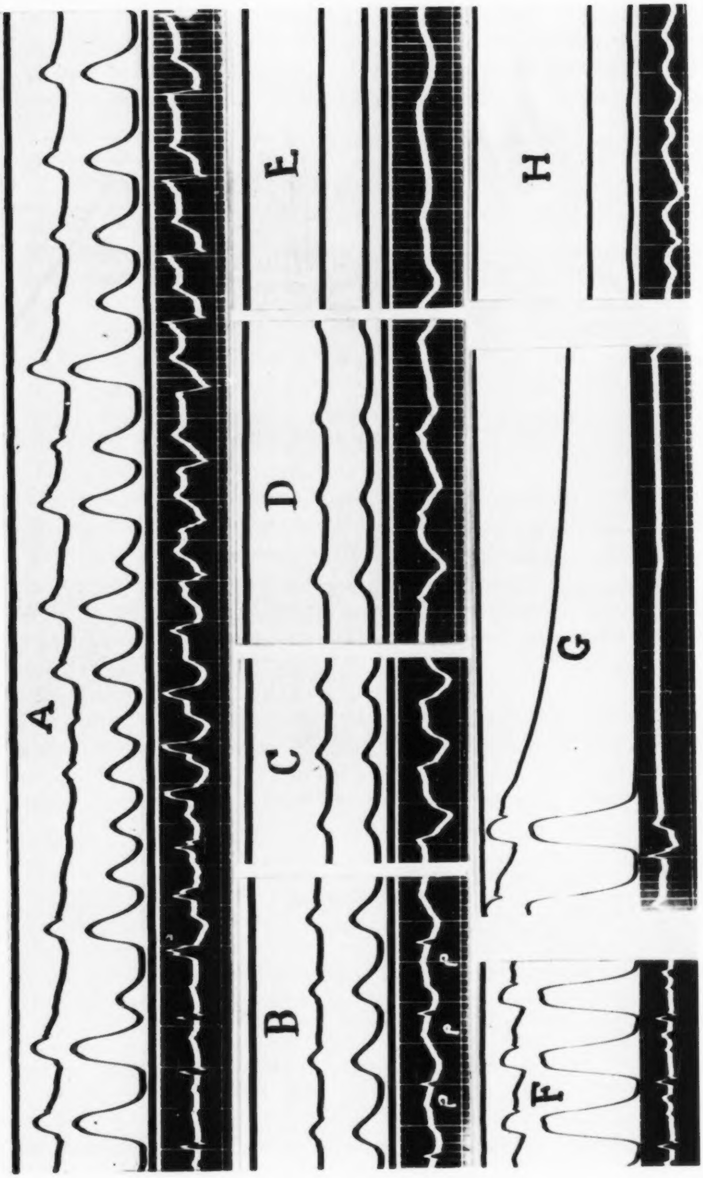

Fig. 2. Aortic and intraventricular pressure curves and electrocardiogram (lead II), illustrating development of fibrillation and one type of diastolic arrest after KCl. A, immediate effects of 42 mgm. KCl per kilo into right auricle; B-E, successive changes leading to complete arrest; (expt. O-83); F-G, from experiment 80, showing F, control; G, prompt diastolic standstill; H, fibrillation on mechanical stimulation

smaller doses, caused fibrillation; in the experiment recorded by figure 2, A-E, fibrillation resulted after rapid injection into the right auricle of a single dose equivalent to 42 mgm. per kilo; and in the experiment illustrated by figure 3, A-C, 30 mgm. per kilo similarly administered proved to be not quite sufficient to produce fibrillation.

Finally, if still larger doses, i.e., higher intra-auricular concentrations, were employed distinct changes in the cardiac mechanism still occurred but fibrillation failed to develop. This is shown in the records of figure 2, F-G and in figure 3, I, H-K.

A careful study of the preliminary stages preceding fibrillation throws some light on the mechanisms responsible for potassium fibrillation. They show a sinus slowing and a depression of A-V conduction, though actual block observed by Mathison was not found in our experiments. Instances of premature ventricular tachysystole, such as reported by Hering (1915) were present in some, but by no means all, of the experiments. They are beautifully shown in the records of figure 2, A and 3, H but are totally absent in the records of figure 1, B and 3, A and B. We conclude that they are incidental phenomena, obviously not an essential prelude to fibrillation as Hering maintained.

The alterations in the electrocardiogram which *uniformly* precede fibrillation, regardless of whether other incidental changes are present or not, are of an essentially different order, and may therefore with greater reason bear some relation to the incoördination that develops subsequently. These changes in the ventricular complexes, which are clearly shown in figures 1, B, 2, A and B, 3, A and B, and 3, I, consist in a diminution of the R deflection, a deepening of the S deflection, a prolongation of the entire QRS complex, a more gradual return to isopotential, followed by a pronounced increase in the final T deflection. In short, the curves display characteristic aberrant deflections probably produced by depression of conduction in the main distributing branches (possibly unequal incomplete bundle-branch blocks). The presence of P waves indicates the maintenance of the beat by a supraventricular focus. The changes are characteristic in all the records, but their gradual development is best followed in the tracings of figure 3, A and B. The first beat in segment A is the only one which is entirely normal. As often happens in the dog, the T wave is negative. Beat by beat S deepens and R becomes smaller. Progressively likewise, the entire QRS complex broadens and T becomes first less negative, then positive. These effects precede slowing of sinus rhythm and prolongation of the P-R interval which remains constant in segment A. The tracing of segment B was taken less than 1 minute later. It represents a more advanced stage of the same phenomenon, P, Q, R, S and T being distinctly obvious in the electrocardiographic records. The P-R interval is somewhat prolonged. Segment C shows the transitional changes.

Coarse, slow peristaltic waves, regular in sequence, were observed to sweep over the ventricles. Their rate increased and observations showed that

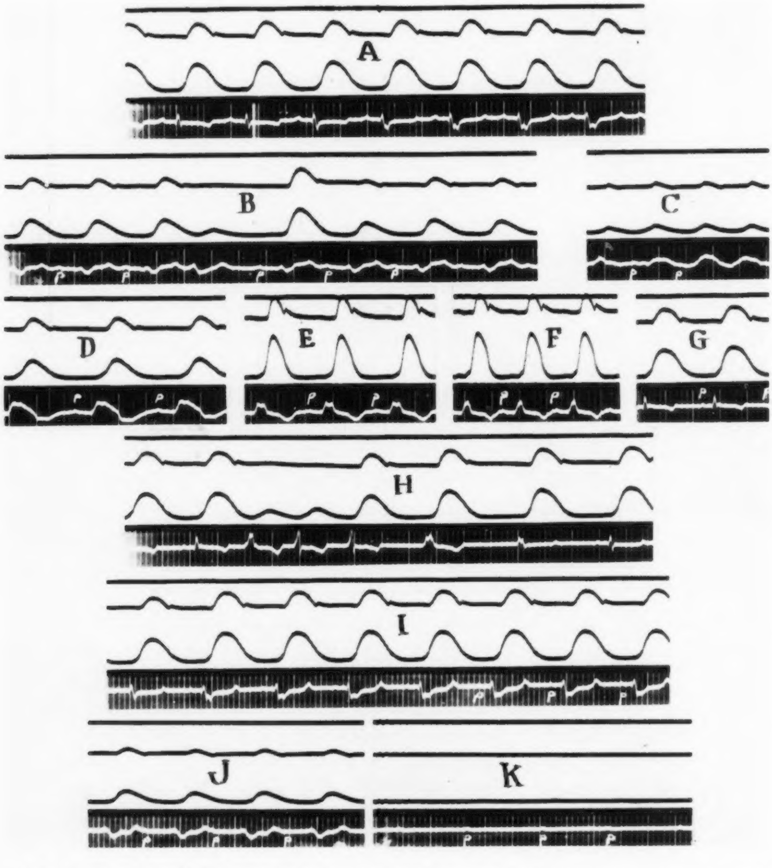

Fig. 3. Aortic and intraventricular pressure curves and electrocardiogram (lead II), illustrating changes in conduction leading to depression and recovery after a first injection and to diastolic arrest after a second. A, immediate effects of 30 mgm. KCl per kilo into auricle; B-C, depressant effects; D-F, recovery; G, normal, 4 min. later; H, immediate effects of a second injection of KCl (50 mgm. per kilo) into auricle; I-J, subsequent effects; K, arrest of a second type.

they were faster than the auricular beats. The waves are therefore probably of ventricular origin. The condition represents a state bordering on incoördination.

The pressure curves in segment A show clearly that in spite of significant alterations in the electrocardiographic complexes, no demonstrable changes occur in the ventricular or aortic pressure pulses. The dynamic action of the left ventricle remains unaltered. Gradually, as shown in segment B, the curves become smaller in amplitude. Measurements demonstrate a prolongation of isometric contraction and shortening of the ejection phase. Even in the stage bordering on incoördination small variations still occur within the ventricle as shown in segment D. In this experiment, full incoördination never developed, the beats gradually returning to normal as illustrated in segments D, E, F and G. The continuation of this experiment is of interest in showing that the same succession of events follows a second larger dose, except that premature contractions are superimposed in segment H. The beat is still supraventricular in origin in segment J, as determined both by direct inspection and by the presence of waves attributable to auricular contraction. The P-R interval is greatly prolonged. In this instance fibrillation also failed to occur, the ventricles coming to diastolic standstill when block became complete, as shown in segment K, in which P waves alone persist.

The transitional changes when incoördination supervenes are illustrated in figure 1, A-E. At the time segments C, D and E were taken the auricles were completely inhibited, the beats being ventricular in origin. The progressive reduction in the size of the pressure curves until no effects are produced is well shown in segment E. At this stage very weak waves of contraction spread over the ventricles.

We conclude from these experiments and others of a similar character that ventricular fibrillation is not a direct consequence of ventricular premature systoles or of ventricular tachysystole, but occurs as a result of depressed conduction affecting first the bundle branches and internal layers of the ventricle and subsequently larger and larger portions of the myocardium. Incoördination follows when some fractions escape complete depression; total cessation occurs when they are completely depressed. Such a diastolic standstill without fibrillation also occurs under other conditions, viz.: 1, when the sinus impulses are completely blocked, as in figure 3, K, the ventricles being incapable of developing an idioventricular rhythm and, 2, when the sinus rhythm is abolished. The tracings of figure 2, F and G, illustrate such a type of stoppage. The cardiac arrest is not unlike vagus inhibition. Ventricular irritability and conduction are not totally depressed, however, for upon mechanical stimulation the ventricles developed an incoördinate beat shown in segment H.

Summarizing our interpretations we may say that diastolic arrest of the beating heart by potassium may be due *a*, to complete A-V block, with depressed idioventricular rhythmicity; *b*, to complete depression of the sinus node, or *c*, to complete depression of irritability and conductivity of

the entire ventricular myocardium. The latter alone is of importance in abolishing fibrillation by potassium. Our results indicate that not only large doses but high concentrations of potassium are required to accomplish this. When lower concentrations are used the probability that fractions of ventricular muscle escape depression is greatly increased, with the result that incoördination develops, or if already present, continues. The necessity of employing concentrated solutions of potassium is thus emphasized.

It remains to analyze the character of the so-called "fibrillation" produced by potassium salts. It differs in many ways from that following faradic stimulation. On inspection of the heart or by viewing moving pictures of the condition it is easily seen that the waves are even slower and coarser than those which characterize the initial stage of fibrillation produced by faradization. The sequence is more regular and each peristaltic wave sweeps over larger sections of the ventricle. Waves traveling at different velocities and frequencies in diverse portions of the ventricular surface are not seen. Only one rhythm is present in the ventricles. The electrocardiographic deflections are also different. This is easily seen by comparing fibrillation, due to potassium, in segments C-F of figure 1 with those brought on by faradization and shown as segments G-J. In the first instance their frequency ranges from 150 to 270 per minute, in the latter from 660 to 1200. This corresponds with direct observations; the highest frequencies observed after K fibrillation are always below the slowest which occur in faradic fibrillation. Their periods and contour may alter slightly from time to time, but the variations are no greater than may be expected from a depressed idioventricular center. The intraventricular pressure curves show that the contractions are at once sufficiently coördinated and strong enough to produce periodic elevations of intraventricular pressure. This is rarely the case in fibrillation following faradic stimulation. It should be added, however, that an exact correspondence is not always maintained. Thus, as shown in figure 1, E, the pressure fluctuations may suddenly cease while the form of the electrocardiogram deflections remains unaltered.

We conclude that the so-called "fibrillation" induced by potassium simulates but is not, in fact, a true incoördination. It is a condition which results from progressive depression of all functions of the heart. By depressing first the conductivity of the His-Tawara system, aberrant contractions are produced and then the control by supraventricular centers is abrogated. A fairly regular idioventricular rhythm is established, but owing to the fact that ventricular irritability is likewise depressed the excitation wave spreads slowly and not always equally through the ventricular myocardium. Not only are fewer and unequal numbers of fractions excited but those that do receive the impulses react only feebly.

In answer to the initial question with which this inquiry started we may

say: The fact that potassium salts produce a condition simulating coarse fibrillation but not actually identical with it is not a contra-indication to the use of potassium salts to abolish an existing fibrillation. On the contrary, the mechanisms involved, viz., depression of all cardiac functions, are precisely the ones we expected to set in operation by the use of potassium for abolishing an existing fibrillation.

The minimal doses of KCl effective in arresting fibrillation. We have previously reported that approximately 1 cc. per kilo of a 5 per cent KCl solution (= 50 mgm. per kilo) is sufficient to arrest the fibrillating ventricles, provided it be administered as divided doses into the two ventricles. The direct experimental evidence upon which these deductions were based is summarized in table 1.[2] The data in column A show that with three exceptions fibrillation continued naturally from 15 to 50 minutes, the average duration being 24 minutes. The shortest interval ever observed was 7.5 minutes. The data in column B show the experiments in which bi-ventricular injections of KCl in different doses successfully abrogated fibrillation in very much shorter intervals, the average of 21 experiments being 2.8 minutes. The minimal dose by which this was accomplished was 40 mgm. per kilo and there is no evidence that larger doses acted more promptly. The data in column C show the experiments in which similar injections failed to abolish fibrillation within the observational periods listed. In 8 of these experiments the dose was less than 50 mgm. per kilo and in 5 experiments, greater. In three of the experiments (O34, O35 and O36) in which large doses failed to arrest fibrillation in less than 5 minutes, the ventricles were massaged immediately after the injections and this also occurred in experiment O28 and O38, in which doses larger than 50 mgm. per kilo caused arrest after 6 minutes. On the basis of these and other observations we concluded that massage delays the action of KCl given intraventricularly. This may conceivably be due to a direct effect of massage on the fibrillating movements or to the dilution of KCl by new blood drawn into the ventricles. The fact that very strong concentrations totally arrest the normally beating ventricles, while somewhat weaker concentrations lead to pseudo-fibrillation, is in favor of the second postulate. In further agreement is the fact that smaller doses of KCl given intraventricularly are not effective in abolishing an existing fibrillation.

We thus arrived at the conclusion that 50 mgm. per kilo mixed with intraventricular blood represents approximately the minimal dose through which the concentration of KCl is raised sufficiently to abrogate an existing fibrillation. Even such doses are not sufficient when the KCl injected into the heart is further diluted, through the influence of massage.

[2] This table by no means includes all experiments which supply probable evidence for our conclusions but only those in which the results could not have been affected by the use of other agents incidentally tested for their ability to arrest fibrillation.

TABLE 1

Factors affecting duration of fibrillation

A SPONTANEOUS CESSATION		*B* CESSATION AFTER BI-VENTRICULAR INJECTIONS OF KCl				*C* INEFFECTIVE BI-VENTRICULAR DOSES OF KCl				*D* CESSATION AFTER RIGHT VENTRICULAR DOSES OF KCl			
Experiment	Duration before arrest	Experiment	Duration previous to use of KCl	Amount per kilo	Continuation before arrest	Experiment	Duration previous to use of KCl	Amount per kilo	Failure to arrest	Experiment	Duration previous to use of KCl	Amount per kilo	Continuation before arrest
	minutes		*minutes*	*mgm.*	*minutes*		*minutes*	*mgm.*	*minutes*		*minutes*	*mgm.*	*minutes*
O20	25.0	O28	5.0	205†	6.0	O34	2.75	56†	7.75	O63	20	40	7.0
O21	17.5*	O29	1.0	62	2.0	O35	2.25	80†	7.0	O64	20	40	12+
O22	19.0	O30	1.75	111	1.75	O36	1.75	70†	30.–	O65	9	42.6	9.5
O23	11.5*	O31	1.5	91	1.5	O45	13.0	40	6.–	O71	4	45.0	18.75
O25	25.0+	O32	1.3	91	4.6	O46	6.25	73	4.5	O72	6	41	5.75
O27	31.0+	O33	3.5	66	1.5	O48	2.0	52	4.25	O73	3.5	105	12.5
O51	50.0	O37	1.25	93	4.75	O59	3.0	43	12.0	O75	4.5	71	9.75
O52	7.5	O38	1.0	86*	5.5	O60	5.0	38	17.0	O76	5.5	73	6.0
O61	9.0	O40	2.0	47	4.0	O66	3.0	25	15.5				
O62	24.0	O41	2.1	46	1.8	O67	2.0	33	11.0				
C450	25.0	O42	3.0	65	2.0	O68	13.0	38	28.0				
C451	26.0		1.25	43	1.25	O69	0.5	30	23.0				
C452	36.0	O43	2.0	45	3.00	O70	2.75	38	12.5				
C454	24.0	O44	2.0	59	2.5								
C455	26.0	O45	3.0	65	2.0								
C456	33.0	O47	3.25	82	3.25								
C457	15.0	O48	1.0	62	1.0								
		O49	3.0	40	5.0								
		O55	1.0	73	2.0								
		O56	1.0	40	2.0								
		O70	1.5	48	1.0								
Range......	7.5–50				1–6				4.25–28				6–18.75
Average.....	24				2.8				13.7				10.3

* Fibrillation due to physical hyperthermia.

† Ventricle massaged.

\+ indicates period longer than that indicated but not actually determined.

The minimal effective total doses (50 mgm. per kilo) are not large when compared to those of Hooker, who suggests 125 mgm. per kilo for intra-arterial injections. Dosages by these essentially different methods are not, however, strictly comparable. In Hooker's intra-arterial method, a large portion of the injected potassium containing solution never reaches the heart through the coronaries; in our procedure a large part similarly fails to reach the myocardium because it is retained within the ventricular cavities and later diluted with the total blood. A marked difference apparently exists, in the concentrations of the KCl solutions, those employed by us being 10 times stronger than those used by Hooker. However, account must be taken of the fact that our solutions were diluted at once by residual blood within the ventricles and that the concentrations reaching more remote portions of the myocardium were probably still further reduced. On the other hand, when solutions are directly introduced into the coronaries the concentration around the individual units of cardiac muscle is practically the same as that of the perfusate.

The distribution of potassium salts through the fibrillating ventricles. Potassium salts introduced directly into the ventricular cavities may conceivably arrest fibrillation through mere contact with the internal layers of cardiac muscle, or they may do so after being distributed effectively to large portions, if not every unit, of fibrillating tissue. The rapidity with which arrest often occurs (less than 2 minutes in 60 per cent of experiments) is in favor of the first view. Were this the case, however, the surface layers would probably respond to artificial stimuli immediately after cessation. Since this never proved to be the case we can infer only that the excitability of the entire ventricular myocardium is reduced, presumably by contact with potassium ions. We can, therefore, reach no other conclusion than that potassium abolishes fibrillation after distribution throughout the heart muscle.

Such distribution without the use of massage may take place in several ways and by operation of a number of different forces, viz.: 1. It may diffuse from the interior through intermuscular spaces or vessels. 2. It may flow into the root of the aorta, enter the coronary vessels and thus reach the ventricular muscle. 3. It may be forced through the Thebesian vessels or coronary sinus, either passively by pressures within the cavities or actively by the massaging effects of the fibrillary contractions. While we may not be in a position to affirm that any of these modes of distribution is chiefly or solely concerned, we have examined the experimental evidence weighing in favor of each possibility.

The rapid diffusibility of KCl into cells and the fact that application of KCl to the surface of the ventricle produces pronounced changes in the electrocardiographic deflections (Wiggers, 1929) favor the first possibility. The fact that other substances, such as $CaCl_2$, epinephrin, etc., introduced

after fibrillation has ceased do not appear to spread readily without massage is opposed to the idea that diffusion is the major force in the distribution of potassium.

The second hypothesis is supported by a number of *a priori* considerations. During fibrillation the left intraventricular pressure is slightly above zero and often irregularly elevated as a result of incoördinate contractions. The left ventricle is visibly reduced in size; hence the probability that some blood (containing KCl) enters the aorta and possibly the coronary vessels. Finally, the mechanical elevation of pressure in consequence of the injection itself may force fluid out of the left ventricle. But a number of experimental facts do not square with this interpretation:—

1. Bi-ventricular administration of effective doses of KCl is equally efficacious after fibrillation is on the wane, and at a time when left intraventricular pressure is practically zero.

2. Massage, which should aid distribution through the coronary system, appears to hinder rather than favor ventricular arrest.

3. Injection of full effective doses of KCl into the left ventricle never abolishes fibrillation without supplementary massage and often fails with massage.

The results of a single experiment may be briefly noted. In experiment O74, 46 mgm. KCl per kilo were injected at 5 per cent KCl solution into the left ventricle, after fibrillation had been in progress for 4 minutes. Seven and one-half minutes later, fibrillation still continued. A second dose of 46 mgm. per kilo was then given. After a lapse of 15 minutes, fibrillation still continued. The ventricles were then gently massaged and after a lapse of another 2 minutes fibrillation had ceased.

4. Fibrillation is still abolished by bi-ventricular injection of effective doses of KCl when the coronary openings into the aorta are occluded by obturators introduced from the aorta.

We demonstrated this in two experiments, one of which may be briefly summarized. In experiment O15 the coronary orifices were cannularized *via* the aorta while the heart was beating. Fibrillation resulted. Five minutes later, 5 per cent KCl solution (64 mgm. per kilo) were injected into the two ventricles. Within $1\frac{1}{2}$ minutes fibrillary activity was very slight and in a total of $4\frac{3}{4}$ minutes it was completely abolished. A normal beat could not be reëstablished by subsequently perfusing 0.2 per cent $CaCl_2$ in isotonic saline through the cannulae inserted directly into the coronary orifices.

We can conclude only that while some of the KCl injected into the left ventricle may be distributed *via* the coronary vessels it is not ordinarily the chief avenue and certainly not an essential route.

The last possibility, viz., that fluid spreads *via* the Thebesian vessels and coronary sinus remains. Pratt (1898), who first demonstrated the

functional importance of these communications, has stated the premises upon which such a mode of distribution may be assumed. He wrote,

> . . . . the heart in the living animal is, during fibrillation, in a state particularly favorable to nutrition through both the vessels of Thebesius and the coronary veins. Measurements taken in the left ventricle show that the intracardiac pressure rises as fibrillation draws near, so that the heart is greatly distended even before it has ceased to beat. The arrest of the ventricle lowers the blood-pressure in the aorta, and hence the blood-pressure in the smallest peripheral coronary arteries, to almost nothing. Consequently, the passage of the blood through the vessels of Thebesius and the regurgitation from the auricle through the coronary veins are doubly aided; on the one hand by the relatively high pressure in the ventricle and auricle, and on the other by the diminished resistance in the coronary vessels. I have already demonstrated how slight a pressure will drive the blood from the interior of the ventricle or auricle through the cardiac walls. The intracardiac pressure at the onset and in the earlier moments of fibrillation would seem to be more than sufficient to establish such a circulation, giving the quivering organ one chance of recovery, although a desperate chance at best.

Furthermore, a number of investigators, Langendorff (1899), H. Fredericq (1908), Hilton and Eichholtz (1924) and Anrep and Häusler (1928) among them, have demonstrated that under equivalent pressures the coronary flow increases as a result of fibrillation. Hammond and Kinosita (1926) attributed this to the massaging effect of the fibrillary contractions, but the results of Anrep and Haüsler appear to demonstrate that the abolition of strong systolic contractions is chiefly responsible. Regardless of the mechanism, it is clear that the resistance to flow in the peripheral vessels of the heart is reduced as a result of fibrillation.

While the physical conditions are ideal for a satisfactory perfusion of the ventricular walls, it is more difficult to obtain positive experimental proof that potassium is distributed in this manner. It occurred to us that this might be tested by limiting injections to the right ventricle, being careful not to massage the heart. If such injections stop fibrillation equally promptly, the *experimentum crucis* would be attained. We investigated this possibility thoroughly, not only to settle the point now at issue but because of its practical importance. Clinically, intracardiac injections into the left ventricle are more difficult than into the right, owing to the small frontal exposure of the left ventricle. Consequently, if injections could be restricted to the right ventricle a distinct practical advantage would accrue. We have already intimated (Wiggers *et al.*, 1930) that this procedure cannot be recommended practically, owing to the long time required to abrogate fibrillation. This in no sense implied, however, that the period of fibrillation cannot be shortened, as is evident from the results recorded in table 1, D. Out of eight experiments in which the total effective doses were administered into the right ventricle, in three instances (O63, O72 and O76) the period of fibrillation was reduced to

intervals less than the shortest normal period shown in division A of the same table, though never to the shortest times noted as a result of biventricular injections. These observations, therefore, offer probable if not crucial evidence that potassium salts are distributed by the Thebesian and venous communications with the cavities of the right heart.

Factors concerned in the revival by calcium of the potassium inhibited heart. We have previously reported (Wiggers *et al.*, 1930) that the fibrillating ventricles of the dog can be revived by subsequent ventricular injections of equivalent amounts of $CaCl_2$ only when this is supplemented by massage. Aside from the unfavorable practical aspects, the fact is of scientific interest. Casually considered, such results make it appear probable that calcium, unlike potassium salts, are not effectively distributed through channels communicating with the interior of the heart, and that they neutralize the effects of potassium only when distributed by the coronary system.

It was difficult to explain this paradoxical situation. We tended at one time to attribute this difference to the fact that the massaging action of fibrillary movements aids in the diffusion of potassium salts and that their cessation was unfavorable to the diffusion of calcium salts injected after fibrillation had ceased. The recent observations of Anrep and Haüsler (1928) indicating that fibrillary contractions exert no massaging effect render this interpretation untenable.

The question then arose whether calcium salts may not be distributed through the ventricles but in insufficient concentrations to neutralize the latter. We must remember that the potassium salts remain within the ventricle, and assuming a continued equal distribution of both salts after calcium injections, the potassium content might still remain in excess around the individual muscle fractions. This conception gains support from experiments recently reported by one of us (Wiggers, 1929). Solutions of $CaCl_2$ introduced into the right ventricle of normally beating perfused hearts of cats, and in such a way that access to the coronary vessels was impossible, produce characteristic stimulating effects in the left as well as in the right ventricle. Unless differences in the dynamic conditions not now apparent exist, it is probable that calcium salts are widely distributed after intraventricular injection.

If this state of affairs exists the virtue of ventricular massage with the expulsion of blood into the aorta and its displacement through the coronary system consists less in distributing calcium salts than in diluting the K containing blood within the ventricles and washing it out of the heart muscle. A number of experimental facts support such a possibility. A perfused heart brought to a diastolic state of rest by potassium can readily be revived by subsequent perfusion with saline solutions containing only normal quantities of calcium. The revival of the beat by perfusion *in situ*

with solutions containing very small percentages of calcium, as practiced by Hooker, undoubtedly owes its success as much to the removal of potassium as to the subsequent use of calcium. Our method of washing out the cardiac vessels with the animal's own blood is admittedly inferior from this point of view, for at best the KCl content is only diluted and not potassium free, as when perfusates are employed. To test this hypothesis further the technique was altered in a number of experiments to the extent that massage was applied before introduction of the $CaCl_2$ solution with the idea of reducing its concentration and washing it out of the heart by a mechanical flow of renewed blood. In one instance this proved so successful that feeble idioventricular beats were initiated without use of $CaCl_2$. Similar results were reported by d'Halluin (1926). In both instances, however, the subsequent use of $CaCl_2$ proved to be necessary in order to start vigorous beats of supraventricular origin.

Our other attempts proved rather disappointing, however, the ventricles reverting to a state of coarse fibrillation. Washing out potassium from the heart by massage before introduction of calcium thus appears to have two opposing effects—one favorable in that it enables subsequent smaller doses of calcium to counteract the effects of potassium; the other unfavorable in that the very act of dilution is apt to restore a fibrillary state. For this reason chiefly we felt it unwise in our earlier communications (1930) to recommend this modification of our technique as a practical measure.

SUMMARY

1. The experiments reported deal with the mechanisms through which successive intraventricular injections of potassium and calcium salts abrogate the fibrillary state and restore a coördinated beat.

2. Doses of KCl smaller than those utilized to arrest fibrillary contractions can produce in the normally beating heart a state resembling fibrillation, but not actually identical with it. The changes preceding and leading up to this condition are analyzed in detail and the so-called "fibrillary contractions" are compared with those resulting from faradic stimulation.

3. We conclude that in this instance the incoördination is the result of depressed conduction, first in the bundle branches and internal layers of the ventricle, and subsequently in diverse portions of the myocardium. Incoördination follows when some fractions escape complete depression, but total cessation occurs when they are completely depressed.

4. The so-called "fibrillation" due to use of potassium salts differs from that following faradic stimulation in the slower, coarser and more regular waves which course over both ventricles. The electrical deflections are larger and more regular while their frequencies (150–270 per minute) are much less than are those which follow after faradic stimulation (660–1200

per minute). The conclusion is reached that the condition produced by potassium is not a complete incoördination.

5. The production of such a condition is in itself no contra-indication to the employment of KCl solution for arresting an existing fibrillation, but emphasizes the importance of employing doses sufficiently large to produce complete depression in all fractions of the heart.

6. The minimal dose that can ordinarily be relied upon to arrest fibrillation is about 50 mgm. per kilo.

7. Evidence is presented that potassium salts administered intraventricularly produce their action after being distributed through the myocardium. This distribution cannot be accounted for by diffusion, nor by an overflow into the aorta and entry into the coronary system. It is chiefly forced through the Thebesian vessels by low but positive pressures within the ventricular cavities.

8. Subsequent injections of calcium chloride fail to restore coördinate beats unless fluid is massaged into the aorta and thus circulated through the coronary system. This is not due to the fact that calcium is not distributed through the walls by the Thebesian vessels as is potassium, but rather to the fact that the potassium is not readily washed out of the tissue unless assisted by massage sufficient to restore a coronary perfusion with the animal's own blood.

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AN APPARENT INFLUENCE OF SYMPATHETIC NERVES ON MUSCLE GLYCOGEN

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That certain parts of the sympathetic system and also organs having a sympathomimetic function may influence the glycogen content of body tissues has long been recognized. Reserves of carbohydrate which are stored in the liver are readily mobilizable by splanchnic excitation, and a similar effect is produced by adrenomedullary secretion. Recent evidence which has been adduced (Cori, 1928–29) suggests, indeed, a noteworthy degradation of muscle glycogen following the administration of adrenalin, although this rather surprising suggestion has not yet been given support (see Cannon, 1929). Recalling the many recent delvings of various workers into sympathetic relationships to muscular activity, it appeared singular that a possible glycotaxic function should be overlooked.

Cats were used in the experiments. To produce sympathetic denervation of the fore-limb muscles, excision was made of the stellate ganglion and the upper portion of the thoracic chain; to treat similarly the muscles of the hind-limb, from 7 to 10 cm. of the abdominal sympathetic trunk were removed. Pflüger's glycogen method was employed. Animals were killed by stunning, and usually within 3 to 5 minutes all the muscle samples used (25 grams of each corresponding muscle) for making the estimations had been excised, minced and introduced into potassium hydrate solution on the boiling water bath. The determinations of blood flow were made as described later.

Negligible variations in the glycogen content of corresponding muscles were observed in normal control animals which were used at the same time as the principal experimental animals. In one instance in which an animal was sacrificed only 11 days after unilateral sympathectomy of the limb muscles, small increments were noted in muscle glycogen on the operated side. In other animals examined a few weeks after operation inconsiderable glycogen differences were noted.

In the majority of the experiments, examination of the tissues was carried out from 70 to over 90 days following the denervation; thus it was hoped to avoid any vascular changes which might follow early after the operation. According to Feldburg (1926) no degenerative changes are observable in

striped muscle up to three months after sympathetic removal. In disagreement, however, is the evidence by Kuré *et al.* (1927), and by Kerper (1928). Comparison of the denervated muscle was made throughout with the correspondingly opposite, normally innervated muscle.

That significant changes are brought about in the glycogen content of long-sympathectomized skeletal muscle is evidenced by the data given in table 1. Chiefly it will be noted that sharp decreases from the normal glycogen percentage occurred in the denervated hind-limb muscles, in all cases. In striking contrast, however, were the considerably augmented amounts of glycogen observed in most cases in the fore-limb muscles on the operated side. Such results did not at first glance appear explicable.

TABLE 1

Changes in muscle glycogen following sympathetic denervation

CAT	SIDE OF ANIMAL SYMPATHECTOMIZED	DAYS KEPT ATER OPERATION	DATE ANIMAL KILLED	CONDITION OF ANIMAL WHEN KILLED	GLYCOGEN OF LIMB MUSCLES IN MGM. PER CENT			
					Fore right	Fore left	Hind right	Hind left
1	Right	86	January 27, 1928	Fasting	470	326	606	642
2	Left	91	January 27, 1928	Fasting	286	550	624	570
3	Right	84	February 1, 1928	Fasting	292	212	312	404
4	Right	77	February 1, 1928	Fasting	270	194	—	—
5	Right	74	February 3, 1928	Well-fed	800	734	674	858
6	Left	72	February 3, 1928	Well-fed	676	740	740	698
7	Right	95	March 6, 1928	Fasting	319	269	309	337
8	*	78	March 6, 1928	Fasting	308	302	243	266
11	Left	79	January 25, 1928	In insulin convulsions	97	90	352	276
12	Left	54	January 25, 1928	In insulin convulsions	182	164	452	292

* Fore left and hind right limbs sympathectomized.

In the majority of the experiments it was found at post-mortem examination, however, that the thoracic sympathetic regrowth had proceeded so far that intimate connection had become established with the cervical nerve trunk (table 2). Besides anatomical, there were also physiological indications of such reconnections, as observed for example in the return towards normal of the ocular (paralytic) conditions. In contradistinction, no regeneration of the abdominal sympathetic chain, to a degree approaching anatomical restitution, was noted in any case. This was largely explainable by the fact that, in order to effect complete denervation, several centimeters of the abdominal sympathetic trunk had been removed at operation. Apparently in relation with the foregoing were the observations, therefore, that in all the experiments considerably smaller amounts

of glycogen were found in the hind-limb muscles on the operated side; whereas in those instances in which thoracic sympathetic reunion with the cervical trunk occurred (with one exception, cat 11, which was killed during severe insulin convulsions) there were marked increases in glycogen percentage in the (previously sympathectomized) fore-limb muscles.

In three instances no change in glycogen content of the fore-limb muscles was noted, and in two of these experiments the thoracic sympathetic reconnections with the cervical trunk were not definitely established. Furthermore there had been induced severe hypoglycemic convulsions, previous to taking the muscle samples for analysis, in two of these animals. No particular consideration will now be given the experiments carried out with insulin; that further light might be thrown on sympathetic function

TABLE 2

Changes in muscle glycogen following sympathetic denervation

CAT	DATE	GLYCOGEN DIFFERENCE IN MGM. PER CENT ON OPERATED SIDE		REGROWTH OF THORACIC SYMPATHETIC
		Fore limb muscles	Hind limb muscles	
1	January 27, 1928	+144	−36	+
2	January 27, 1928	+264	−54	+
3	February 1, 1928	+80	−92	+
4	February 1, 1928	+76	—	+
5	February 3, 1928	+66	−184	+
6	February 3, 1928	+64	−42	+
7	March 6, 1928	+50	−28	?
8	March 6, 1928	−6	−23	?
11	January 25, 1928	−7*	−76*	+
12	January 25, 1928	−18*	−160*	?

* After severe insulin convulsions.

by introducing an emergency situation, such as severe hypoglycemia, was at the time thought probable. A summary of the data is shown in table 2.

It appeared possible that the results might be due to circulatory differences, although the experiments were carried out many weeks after the preliminary surgical procedures had been performed. Wastl (1924) indicates that sympathetic nerves may affect striated muscle by modifying the circulation, and Tower (1926) also presents evidence of a quantitative relationship between blood flow and muscle tonus. The distribution of non-medullated nerve fibers to the vessels of skeletal muscles has been considered by Kerper (1927). Determinations of blood flow through the fore and hind limbs were therefore carried out on several animals at various periods after sympathectomy.

Light ether anesthesia was induced, and simultaneous collections and

careful weighings were made of blood samples taken from veins at corresponding points on each side. It will be observed (table 3) that blood flow through the fore limb on the operated side was markedly increased in one animal, cat P, which was sacrificed only 24 days after operation and showed no thoracic sympathetic regrowth. Relatively small circulatory differences in the fore limbs were observed, however, in those cases in which partial or extensive regeneration of the thoracic trunk had taken place. In significant contrast the blood flow through the hind limbs was in all instances greater—usually much greater—on the sympathectomized than on the normal side.

It has been stated by some authors that the circulatory changes produced by removal of the sympathetic nerve supply tend to disappear early (Lee, 1929), and commonly within a relatively short period (i.e., a few

TABLE 3

Blood flow through fore and hind limbs following sympathectomy

Cat	Date of experiment	Number of days after sympathectomy	Side of animal sympathectomized	Blood flow (grams per minute): Fore limb: Right axillary vein	Fore limb: Left axillary vein	Fore limb: Difference on operated side	Fore limb: Per cent increase on operated side	Hind limb: Right femoral vein	Hind limb: Left femoral vein	Hind limb: Difference on operated side	Hind limb: Per cent increase on operated side	Thoracic sympathetic regrowth
P	August 8, 1929	24	Left	4.21	9.86	+5.65	134	1.07*	1.48*	+0.41	38	Absent
Q	August 8, 1929	62	Right	6.77	5.75	+1.02	18	0.58	0.15*	+0.43	281	Extensive
R	August 9, 1929	60	Right	8.20	6.08	+2.12	35	45.12	26.54	+18.58	70	Partial
S	August 9, 1929	26	Left	4.18	5.03	+0.85	23	4.80	12.78	+7.98	166	Partial

* Blood flow through lower portion of femoral vein *ex* profunda branch; other determinations *include* profunda vein blood.

weeks or so), after operation (Tower, 1926; Coates and Tiegs, 1928; Kuntz, 1929). Dependency of vascular recovery on at least partial regeneration of the autonomic supply would seem to be indicated, however, by the present study. Very clearly circulatory defects incident to ablation of considerable lengths of the abdominal sympathetic trunk were present many weeks after operation. The extra-muscular vasculature of the limbs may conceivably suffer greatest from sympathetic excision; Langley (1900) arrays evidence which points, indeed, to slight effects only on blood flow through limb muscles being produced by excitation of autonomic nerves. Muscle glycogen variations indicated herein would not therefore appear to be referable to the circulatory changes described.

While the present work was in progress a report by Hofmann and Wertheimer (1928), which dealt with the effects of sympathetic nerves on

muscle glycogen in frogs, was observed. These investigators used their animals only a few days after the operations had been carried out, and noted that ordinarily in the gastrocnemius and vastus muscles there were no appreciable changes after sympathectomy. After strychnine or after adrenalin injections, more glycogen was found on the operated than on the normal side. Büttner also has reported (1929) the presence of increased amounts of glycogen in skeletal muscle following excision of the sympathetic supply.

SUMMARY

In the chronic condition, sympathectomized muscle appears to suffer a diminution of its normal glycogen content.

If removal of the sympathetic supply to a limb is followed, however, by re-establishment of the nervous connections, there occurs an augmentation of the glycogen store in the muscle as compared to that on the unoperated side. This increase may be the result of hyperactivity of the regenerated sympathetic nerves.

The blood flow through the sympathectomized fore limbs in animals killed one to two months after operation was found to correspond approximately to the normal, but was much greater than normal through the operated hind limbs. Reunion of the nerve trunk in the former cases and non-union in the latter probably explain the circulatory differences. The muscle glycogen changes do not appear to be dependent alone (if at all) on vascular shifts.

It is recalled that changes in glycogen equilibrium in tissues may be brought about by certain sympathetic (splanchnic) and also by medulliadrenal (sympathomimetic) agencies. In keeping with these activities the peripherally-distributed sympathetic nerves now appear to subserve a glycotaxic or glycogenotaxic function in skeletal muscle.

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THE EFFECTS OF X-RAYS ON THE ADRENAL GLAND

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Various glandular tissues are known to be very sensitive to x-rays. In general the reactions produced are atrophy and autolysis with proliferation of connective tissue. This has been demonstrated experimentally for the intestinal mucosa (1), (2), the gastric mucosa (3), (4), the kidney (5) and the pancreas (6). Relatively little is known as to the effects on the adrenal gland. A number of clinical writers have suggested that certain functional disturbances following abdominal irradiation might be due to altered adrenal function, although there is little supporting evidence. Des Jardiens (7) has recently reviewed the literature on this subject.

Lacassagne and Samssonow (8) implanted radium in the adrenals of a series of rabbits, and found resulting necrosis of the glands. In one series of animals, death followed in 17 to 35 days; in another series, in which the necrosis was confined to the medulla of the gland, the animals survived without symptoms until sacrificed, 4 to 7 weeks later.

Wislocki and Crowe (9) carried out similar experiments in which radium was implanted in the adrenals of dogs. They also reported that necrosis of the gland occurs, but that if a fragment of the cortex remains, the animals survive although all traces of the medulla have disappeared. A report of preliminary experiments on the effects of x-rays on the isolated adrenal gland of dogs was published in 1924 by Martin, Fisher, and Rogers (10). Since that date, Fisher, Larson and Bachem (11) have continued the work and reported gross degenerative changes of the adrenal following exposure to x-rays This was associated with the appearance of asthenia, diarrhea, depression, and death.

Our preliminary work indicated to us the need of a more intensive study than was first planned. This first work was inadequate and merely indicated the following facts: that a relatively high intensity of irradiation would be required to induce deficiency effects; that a specific study of the pathological histology of the irradiated gland was needed; and that the possible onset of deficiency effects was slow and would require the lapse of several months after irradiation, to become manifest.

During the last two years, we have been continuing this investigation

with the purpose of gaining information 1, of the histologic changes induced by x-rays applied directly to the exposed and isolated suprarenal gland, and 2, to determine whether or not functional changes or suprarenal deficiency effects could be induced by such irradiation.

Methods. The surgical technique employed in this work was devised by Dr. N. F. Fisher; Dr. C. L. Martin devised the method and administered the x-rays to the glands.

A series of 16 female dogs was used in this study. The right adrenal was removed through an anterior incision through the right rectus. As is well known, if properly done, the removal of a single adrenal produces no serious results in the healthy animal. Three or four weeks later when this initial wound had healed and the dog was obviously in good health, the x-ray dose was applied to the left adrenal in the following manner. With ether anesthesia, a left rectus incision was made and the left adrenal exposed and the surrounding viscera were packed away from the gland with gauze. A hollow truncated cone of nickel plated copper was then lowered directly over the exposed gland. The lower opening was 5 cm. in diameter. The thickness of the wall of the cone was 0.5 mm. The upper end of the cone was fitted by a broad metal plate to the x-ray tube stand. The lower end of the cone was fitted directly over the gland after pushing aside the kidney, spleen, intestine, and stomach. These viscera were packed aside with gauze to prevent contact with the metal cone. The cone was pressed down against the posterior peritoneal wall of the abdomen, thus enclosing the isolated adrenal. Varying dosages of x-rays were applied to the gland in different animals as indicated in table 1. It will be noted that practically unfiltered x-rays were employed in these tests. A piece of thick writing paper was usually employed as a filter to exclude heat effects from the exposed gland. In two experiments, aluminum filters of 0.2 mm. thickness were used. After administering the x-rays the abdominal incision was closed and the animals kept under observation for varying lengths of time. Specific efforts were made to select healthy dogs for this work; to avoid distemper; and to provide an adequate diet. Part of the time the dogs were in cages, but much of the time they were free in a large open runway.

Blood sugar and blood chlorides were determined on the animals at weekly intervals, and in some cases, basal metabolic readings at intervals of seven to ten days. Body weight and temperature, nutritional condition, and spontaneous activity were watched closely. Certain animals were sacrificed at varying time intervals with immediate autopsy and transfer of the irradiated gland to fixing solution for microscopic study. It is well known that after death, the suprarenal medulla quickly undergoes autolysis. All histological statements made in this report are based on glands removed in less than thirty minutes after the death of the animal.

The greatest technical difficulty specifically encountered was in isolating

the gland for irradiation. A loop of intestine or a portion of the stomach may easily slip beneath the edge of the cone into the irradiated area. The left border of the vena cava may be included in the zone. Downward pressure of the cone sufficient to exclude all the surrounding organs may traumatise the vena cava or cause thrombosis of the lumbro-adrenal vein which crosses the irradiated zone. It is impossible in the present work to exclude thrombosis following mechanical injury to these veins, as a contributing cause to the effects described.

The intensity employed in the different experiments is given in table 1. No attempt to express the doses in terms of E units is made since a reliable

TABLE 1

Intensity and estimated x-ray doses applied directly to the exposed adrenal gland of experimental animals

NUMBER OF DOG	PEAK	MILLIAMPERES	FILTER	TIME	TARGET DISTANCE	ESTIMATED MINIMUM HUMAN ERYTHEMA DOSES	LENGTH OF LIFE AFTER IRRADIATION
	kilovolts			*minutes*	*inches*		*days*
1	72	10	Writing paper	7.5	10	3.5	45 Sacrificed
2	72	10	Writing paper	7.5	10	3.5	58 Sacrificed
3	72	10	Writing paper	7.5	10	3.5	Living after 2 years
4	87	10	Writing paper	10	10	6.5	42 Sacrificed
5	99	10	Writing paper	10	10	8.0	12 Died of distemper
6	95	10	Writing paper	10	10	7.5	35 Sacrificed
7	99	10	Writing paper	10	10	8.0	42 Sacrificed
10	140	6	0.2 mm. aluminium	30	10	14.4	78 Sacrificed
11	140	6	0.2 mm. aluminium	30	10	14.4	80 Sacrificed
12	140	6	Writing paper	40	10	19.2	143 Died
14	140	6	Writing paper	40	10	32.0	23 Died

apparatus for measuring unfiltered rays by the ionization method was not available. The x-ray machine was being used routinely for clinical therapy at the time the experiments were in progress and the computation of the doses in terms of human minimum erythema doses was therefore possible. A faint but definite reddening of the human skin appearing in about ten days after treatment was given was used as a unit erythema dose.

Results. The work was done on 16 dogs using varying amounts of x-rays and keeping the animals varying periods of time for observation. This report summarizes the results observed on nine of these dogs. Results on the remaining seven are not cited for various reasons as they were

seen to be inapplicable. Two animals died of distemper. In two dogs, autopsy showed that a portion of the right adrenal had not been removed at the initial operation; in one instance, it was found that an isolated lymph node had been irradiated instead of the adrenal; and in a few cases the animals were sacrificed shortly after irradiation to observe the degree of abdominal and intestinal traumatism involved in the mechanical processes essential to irradiation.

Histological changes of the irradiated gland. We observed early in the course of this work that the suprarenal gland must be excised immediately after death if post-mortem autolysis is to be prevented. The following description is based on those glands which were removed within a few

Fig. 1. Photomicrographs of normal adrenal of dog and the adrenal of dog 12 which had been exposed to 19 human minimum erythema doses, 4½ months before death. It will be observed that there is extensive fibrosis in the entire gland with almost total atrophy of the medulla, zona glomerulosa, and zona reticularis, and extensive disorganization of the zona fasiculata.

minutes of cessation of breathing and the changes described are therefore ante-mortem.

The glands were fixed in Zenker's solution and stained with hematoxylin and eosin, using uniform technique throughout the series. The detailed histological findings of 9 irradiated glands are summarized in table 2. The principal histological findings at the time of death were the following: In all cases there was a thickening of the fibrous capsule of the gland. This was greater on the anterior or peritoneal surface directly exposed to the irradiation than on the posterior surface. Fibrous proliferation extended into the gland to a varying degree according to the intensity of the radiation, being slight with intensities up to ten human erythema

doses and very marked with dosage of 14 to 19. Otherwise there were only slight changes in the structure of the gland, if the intensity of the radiation did not exceed ten erythema doses.

With intensity of 14 or more, marked changes in structure were produced. This consisted in fibrosis of the medulla with actual disappearance of the medullary cells; with fibrous proliferation in the cortex and with the disappearance of the zona glomerulosa and zona reticularis (fig. 1). The zona fasiculata was most resistant to radiation and maintained its histologic structure in all animals (with one exception). Vacuolation, pyknosis, and disorganization was particularly marked in dog 12. There was a gross

TABLE 2

Summary of the histological changes in the adrenal gland following application of varying dosage of x-rays

Positive and negative findings are indicated by + and − signs. Double and triple signs indicate relative severity of the process. For dogs 11 and 12, two columns are included; *a* indicates the findings in the anterior portion of the gland directly exposed to the radiation, *b* indicates the posterior portion of the gland.

	DOG 6	DOG 7	DOG 8	DOG 9	DOG 10	DOG 11-a	DOG 11-b	DOG 12-a	DOG 12-b
Erythema doses	8	8	12	10	14	14		19	
Fibrous thickening of capsule of gland	+	+	+	+	+	++	+	+++	++
All three zones of cortex present	+	+	+	+	+	−	−	−	−
Zona glomerulosa present	+	+	+	+	−	−	+	−	−
Zona fasiculata present	+	+	+	+	+	+	+	−	+
Zona reticularis present	+	+	+	+	+	−	−	−	−
Fibrosis of cortex	−	−	−	+	+	+	+	++	++
Fibrosis of medulla	−	−	−	−	+	+		++	
Normal arrangement of medullary cells	+	+	+	+	+	−		−	
Marked reduction in size of medulla, atrophy	−	−	−	−	−	+		+	
Gross edema, thrombosis, and cellular disorganization of entire gland	Dog 14								

edema of the entire gland with complete disorganization of the gland in dog 14 which received radiation equal to 32 human erythema doses. In this case there was obvious thrombosis of the lumbo-adrenal vein. This animal lived only 24 days after irradiation.

We believe that if several of the animals had been kept for a period of time longer than three months, a greater amount of change would have been evident. Excessively large doses seem to produce a slowly progressive atrophy of the gland with an accompanying fibrous tissue proliferation.

Physiological effects. We had expected to find the suprarenal gland rather sensitive to irradiation and that the degenerative changes would appear rather quickly and hence in our first experiments used a dosage of

from 3 to 6 erythema doses. After repeated attempts we found that both these assumptions were erroneous; that it requires heavy dosage and a long period of time before gross histologic changes and deficiency symptoms appear (see table 2). Of the entire series of experiments, in only two dogs did we observe deficiency symptoms; namely, in dogs 12 and 14. In both of these animals excessively heavy dosage was employed. But we have not excluded the possibility that if some of the dogs which were sacrificed had been kept for a period of time longer than three months, deficiency symptoms might have appeared. This is particularly true of dogs 10 and 11, the glands of which exhibited histologic changes but unfortunately were sacrificed before symptoms appeared. In 8 dogs which received from 6 to 14 erythema doses and were sacrificed in from 4 to 11 weeks no symptoms attributable to suprarenal deficiency could be detected. These animals lived in good health, maintained normal playful activities, exhibited no gastro-intestinal disorders, no alteration of blood sugar, blood chlorides or basal metabolism, no loss of weight, no depression of body temperature, no signs of weakness or lethargy. Dog 3 received three and one-half erythema doses and is living in excellent health two years after the irradiation. However, in the case of two animals, 12 and 14, certain definite and characteristic symptoms appeared and these will be described in detail. The history of no. 12 we think is the most significant of the entire series.

Dog 12. November 8, 1928. Removed the right adrenal; recovery uneventful.

December 9, 1928. The left adrenal was irradiated (19 erythema units).

January 24, 1929. Dog in good condition; weight 37 lbs.

February 25, 1929. Dog in good condition.

March 7, 1929. Dog appears depressed. Vomiting; refuses food. Weight 34 lbs.

March 9, 1929. Dog appears normal; playful and appetite good.

March 25, 1929. Dog appears normal; weight 33 lbs.; temperature 100.5 degrees.

April 8, 1929. Dog appears normal; weight 31 lbs.

April 17, 1929. Dog is rather quiet; weight 28.5 lbs.; no diarrhea or vomiting; temperature 99.6 degrees.

April 29, 1929. Dog is very weak and unsteady in its gait. Prefers to lie quietly in cage. Diarrhea. Weight 27 lbs.; temperature 99.0 degrees.

May 1, 1929, 10 a.m. Very weak; refuses food; weight 26 lbs.: temperature 98.6 degrees.

May 1, 1929, 4:30 p.m. Dog died, immediate autopsy.

Autopsy. The dog is somewhat emaciated. The lungs are clear. There is no pus nor evidence of distemper in the chest. The pancreas is markedly hyperemic and the mucosa of the small intestine exhibits numerous small hyperemic and petechial spots. The kidneys appeared normal. There was scar tissue at the site of the right adrenal but no glandular tissue. At the site of the irradiated left adrenal was a small nodule covered by fibrous tissue, which weighed 420 mgm. This nodule was examined microscopically after fixation in Zenker's solution and staining in hematoxylin and eosin (see fig. 1). Histological examination revealed extensive fibrosis of the entire gland. The medulla had almost completely disappeared and the zona glomerulosa and zona

reticularis had been completely replaced by fibrous tissue with considerable distortion of the area fasiculata.

During the last thirty days of life there was a slight but distinct depression of the blood chlorides, oxygen intake and carbon dioxide excretion.

About four months after heavy irradiation this animal began to exhibit the following symptoms; gradual onset of asthenia and inactivity, loss of body weight, diarrhea, a depression of the blood chlorides, basal metabolism, and body temperature. At no time did this dog suffer from distemper with cough or purulent discharge from nose or eyes. This animal exhibited the syndrome of progressive asthenia and depression of metabolism associated with fibrosis of the irradiated gland and with degeneration of the medulla and of a large portion of the cortex, particularly the areas glomerulosa and reticularis which had completely disappeared.

Dog 14. January 5, 1929. The right adrenal was removed; recovery uneventful.

February 3, 1929. Irradiation of the left adrenal (32 erythema doses).

February 9, 1929. Dog seems to be in good condition; appetite good.

February 12, 1929. The animal appears to be weak; gait is slow and staggering but appetite good.

February 14, 1929. The dog lies quietly in its cage; is somewhat depressed but appetite is good. Fights another dog for food.

February 24, 1929. Inactive; refuses food; is very weak. There is a bloody diarrhea, but no cough. Can walk only a few steps, then sinks to the floor.

February 25, 1929, 10 a.m. Refuses food; staggering gait; is very weak and sluggish. Bloody diarrhea. Temperature 99 degrees.

5 p.m. Prostration. Temperature 96.5 degrees.

February 26, 1929, 1 a.m. Drinks water but is unable to stand on feet. Temperature 96.0 degrees.

7:30 a.m. Dog died.

Autopsy. The left adrenal gland is grossly enlarged and edematous and is surrounded by a very dense, tough, fibrous capsule. Weight 3.1 grams, length 3.5 cm. There are many adhesions between the stomach, spleen, and parietal wall in the region of the left adrenal. There was gross congestion of the spleen; multiple hyperemic and hemorrhagic patches in the mucous membrane of the small intestine, but no open ulcers. The lumen of the gut contains much mucus and blood stained material. The kidneys appear normal. The lungs are clear; there is no pus in the bronchi; no evidence of distemper. The left lung contains an area of hypostatic hyperemia, the right lung is clear.

Microscopically, the adrenal is grossly edematous with fading of the cellular borders and loss of staining power of all portions of the gland.

This animal, which received the excessive dosage of 32 human erythema doses, exhibited the same picture symptomatically, and at autopsy gave findings closely resembling those described by Stewart and Rogoff in acute adrenal deficiency.

SUMMARY

The direct application of x-rays of an intensity estimated as three and one-half human erythema doses to the one exposed adrenal gland of dogs,

after the excision of the other gland, produced no subsequent changes in the gland except a slight fibrosis. No functional disturbances appeared in these animals during a period of observation of from three to twelve months after the irradiation. The application of six to eight doses to the adrenal gland produced no observable functional disorders in dogs kept three months after irradiation, although fibrous proliferation in the gland can be demonstrated microscopically.

Adrenal deficiency symptoms can be induced in dogs by the surgical excision of one gland and giving excessive x-ray doses to the remaining gland, but the lapse of several months may be required for their appearance. These effects are as follows; the gradual onset of a progressive muscular weakness, depression of metabolism, and terminal lowering of the blood chlorides and death.

Heavy dosage of x-rays to the exposed adrenal gland induces degenerative changes, first in the medulla of the gland, and then of the zona reticularis and zona glomerulosa and an extensive proliferation of fibrous tissue throughout the gland.

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OBSERVATIONS ON THE CENTRAL CONTROL OF SHIVERING AND OF HEAT REGULATION IN THE RABBIT

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Shivering may be defined in the words of Richet (1893) as a generalized involuntary trembling of the body muscles, convulsive, rhythmic in character, accompanied by a sensation of cold. According to this author there are three types of shivering—thermal, toxic, and psychic. Thermal shivering occurs in response to cold. Toxic shivering is seen during the chill associated with many systemic infections. Psychic shivering is observed during emotional stimulation, as fear or rage, and when very marked resembles thermal shivering closely.

During vigorous shivering practically all of the skeletal musculature is involved, the tremors occurring at the rate of 8 to 12 per second (Richet, 1893). This increased muscular activity may proceed for hours without obvious diminution in intensity. Shivering is therefore one of the most widespread and vigorous reactions observed in the higher animals. Yet, apart from its influence on heat production, it has interested few investigators; its precise significance in heat regulation is still not settled, while very little indeed is known concerning its mode of origin and its nervous control.

Richet (1893) pointed out that in the cooled animal trembling may occur under two different sets of conditions. In many animals shivering may immediately follow exposure to cold, before any demonstrable fall in body temperature. This immediate reaction Richet calls "reflex shivering," the adequate stimulus travelling presumably by way of the nerves from the cold spots to a nervous centre. Under anesthesia, however, shivering is always preceded by some fall in body temperature, and a deeply anesthetized cat or dog may not shiver until its body temperature has fallen as much 6°C. This delayed response Richet considers to be the result of direct cooling of the centre; hence his term "central shivering."

Regardless of the mode of onset, Richet believed shivering to be under control of a centre. Thus, in a shivering dog, he transected the spinal cord at the level of the first thoracic vertebra. Several minutes later, rhythmic tremors were observed in the muscles innervated by the cervical

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cord; there was no evidence of shivering in the lower body muscles. Additional evidence in favor of shivering as a central phenomenon was brought forward by Sherrington and Laslett (1903), and by Sherrington (1924). The latter immersed the insentient (paraplegic) region of a dog in ice-water, 19 months after spinal transection at the level of the eighth cervical vertebra. Shivering appeared in the head and neck muscles, but not in the muscles innervated from the portion of the cord below the point of section. There is thus strong experimental proof for a supraspinal control of shivering.

The exact localization of this shivering centre has, as far as the writer is aware, not been determined. More than fifty years ago Goltz (1874) stated that the shivering reflex, stimulated through cooling of the blood, must have its centre in the medulla oblongata. Richet (1892) from analogy with his polypnea centre also considered it to be in the medulla oblongata. Sherrington (1924) suggested that it may correspond with the centre for general heat regulation, in view of the fact that shivering from cold is "an item of general thermotaxis." In favor of this possibility are the researches of Bazett and Penfield (1922) and of Rogers and Lackey (1923). The former investigators found all evidence of heat regulating capacity absent in their chronic decerebrate cats, and observed no shivering in these animals. In the pigeon, Rogers and Lackey reported that destruction of the optic thalami was incompatible with shivering or other means of increased heat output in response to cold. The work of Isenschmid and Schnitzler (1914) indicates that in the rabbit heat regulation is controlled by centres in the vicinity of the tuber cinereum. They make no mention of the effects of injuries in this region upon shivering.

In the present research an attempt has been made to arrive at a more precise localization of the central control of shivering. The experiments to be reported were performed on rabbits. All the experiments were acute, consisting of ablation of part of the brain stem and subsequent observation of the animal's ability to shiver in response to cold.

METHODS. After anesthetization with amytal[2] or ether, all the operative steps except the actual brain incision were rapidly completed. The animal was then placed on a warming pad for a time, to allow some measure of recovery. Shivering was then induced by exposing the animal to cold. When the shivering had become generalized and vigorous, the brain was

[2] While 0.5 to 0.6 cc. of a 10 per cent solution of amytal per kgm. intraperitoneally will produce long-lasting surgical anesthesia in the cat or dog, a much larger dose is required in the rabbit. Even then the anesthetic effects are apt to wear off within 1 or 2 hours. Intravenous administration, in doses of 0.3 to 0.4 cc. of a 10 per cent solution per kilogram was found to produce relatively deep anesthesia in most rabbits. Usually, further injections at the rate of about 0.2 cc. per hour were required to keep the animals quiescent. There seems to be considerable variation in susceptibility to this drug among rabbits.

cut. Since a clean, complete transection was aimed at, a strip of razor blade was used in the early experiments, but the resulting hemorrhage was usually great and the shock profound. It was found that a complete separation with much less bleeding could be effected by the manipulation of a small, slender scalpel, rounded at the point and dulled along the cutting edge by use of a stone. Some time after the incision, when the condition of the animal was judged to be sufficiently recovered, the shivering capacity was tested by exposure to cold. At the termination of the experiment the animal was killed and the brain carefully removed and placed in alcohol. Some weeks later when sufficient hardening had occurred to permit of handling, the brain was examined and the transection level determined. All the observations reported here have been made on animals in which the brain stem was found to be completely or virtually completely divided. A considerable number of results obtained after incomplete transection have been discarded.

Shivering in a rabbit under amytal anesthesia may be elicited either by exposing the animal to severe cold (e.g., placing it in front of an open window in mid-winter) or by lowering its body temperature slowly in a cool room. The amount of cooling required depends on the depth of the anesthesia. Under light amytal, shivering may supervene within an hour, when the body temperature has fallen not more than 3°C. In deeply anesthetized animals shivering is usually preceded by a drop in body temperature as much as 7°C. Once started, in the intact animal shivering continues for hours with well maintained intensity.

In a rabbit whose brain has been injured, shivering is much more difficult to elicit. Furthermore, it is usually less intense, as measured by the amplitude of the movements and the number of muscles involved. Often the post-operative response to cooling consists of slight muscular tremors, affecting the upper or lower limbs. These tremors were regarded as shivering only when they were seen to be related to cold stimulation, and were rhythmic and bilateral.

In the course of the earliest experiments it became obvious that in a research of this type negative results are of little value. All general anesthetics depress shivering to an extent roughly proportional to the dosage. Furthermore, after shivering has been induced under anesthesia, in many cases it stops during the preliminary operative steps, even before the brain is cut. Thereafter the animals can be cooled to a temperature incompatible with life, without a sign of shivering. In many instances it was found, too, that shivering may cease and never return after an injury to any part of the brain, even the frontal lobes. In view of the attendant difficulties, when shivering disappeared after a transection, the experiment was repeated; often shivering was elicited after section at a level that had previously given negative results. This mode of procedure

called for the use of a rather large number of animals, but produced a not inconsiderable number of positive results. There is little question that in any research involving ablation of the brain stem a single positive result is more significant than a number of negative findings.

RESULTS. The conclusions brought forward in this paper are based on 21 positive observations, out of a total of 65 experiments. In these 21 instances typical or fairly typical muscular activity in response to cold could be evoked after section of the brain stem at the levels described below. In a considerable number of the remaining experiments shivering occurred after partial brain transections; these results are not included. The rest of the experiments were negative; some of the animals died soon after the operation, while the others ceased shivering immediately after the brain incision, and remained inactive in spite of prolonged cooling.

Of the 21 observations reported, 14 were obtained after amytal anesthesia, 7 after ether. The various transection levels are given in table 1. Photographs of some of the brains appear below.

Shivering after removal of the cerebral hemispheres. That strong thermal shivering may be evoked after removal of both cerebral hemispheres is shown by experiment 39. In this rabbit the brain lobes were extirpated in the manner described by Bard (1928). Dorsally, the transection passed immediately in front of the optic thalami; ventrally it was about 2 mm. in front of the optic tracts. The animal shivered as well as a normal rabbit, and was able to raise its body temperature 1.8°C. in 50 minutes. The protocol follows:

February 11, 1929. Rabbit 39. Weight, 1.7 kgm.

10:10 a.m.	Given 0.3 cc. amytal per kgm. intravenously—both common carotid arteries ligated—skull trephined over left hemisphere.
1:45 p.m.	Rabbit shivering vigorously after exposure to cold—rectal temp. 34°C., room temp. 23°C.
4:10 p.m.	Rectal temp. 35.6°C., room temp. 23.3°C.—strong shivering. Trephine hole enlarged to expose both hemispheres—these were quickly removed by the use of a curved, blunt instrument—comparatively little hemorrhage, arrested by intermittent compression of vertebral arteries. Shivering greatly diminished at end of operation.
5:00 p.m.	Shivering vigorously—rectal temp. 37.4°C.
6:00 p.m.	Animal killed, brain removed.

Shivering after transections through the diencephalon. According to Winkler and Potter (1911, 1914), the diencephalon is that part of the brain limited in front by the anterior commissure and the optic chiasma, and cauded by the epiphysis and the corpus mammillare; it contains the thalamus and the hypothalamus. That this portion of the brain stem is not essential for thermal shivering was shown in four experiments. The sections passed through the levels indicated below.

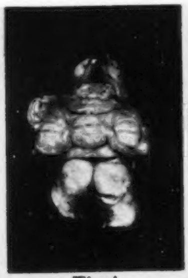

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Fig.7

Fig.8

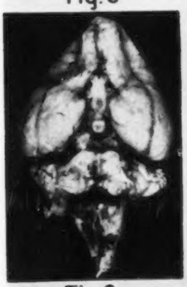

Fig.9

Fig.10

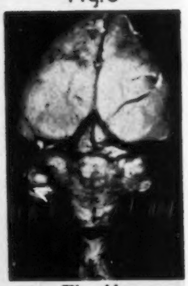

Fig.11

Rabbit 27, transection 2—through the anterior portion of the thalamus, and behind the optic chiasma.

Rabbit 31, transection 3—through the anterior portion of the thalamus and the middle of the corpus mammillare (fig. 1).

Rabbit 27, transection 3—in front of the anterior colliculi, and through the posterior part of the corpus mammillare.

Rabbit 30. Same as 27, 3.

The protocol of one of these experiments (rabbit 27) is given below. There is little question that the capacity for shivering is greatly reduced as the result of injury to this part of the brain. It was in this region particularly that the number of negative results far exceeded the positive experiments, so that, on the basis of a limited number of observations, one might easily be led to believe that shivering is impossible after injury to, or absence of, the diencephalon. The matter is settled beyond all doubt by the results not only of these, but of subsequent experiments, in which shivering occurred after transection at a much lower level.

December 14, 1928. Rabbit 27. Weight 2.11 kgm.

9:30 a.m.	Amytal anesthesia—left carotid artery ligated.
6:30 p.m.	Exposed to cold draft for 15 minutes—vigorous shivering induced—right carotid artery ligated—skull trephined—shivering not affected.
7:00 p.m.	Transection 1—shivering present after completion. This section passed through the frontal lobes.
7:20 p.m.	Transection 2—shivering stopped, but returned markedly in 5 minutes. This cut passed through the anterior portion of the thalamus and just behind the optic chiasma.
7:40 p.m.	Transection 3—shivering stopped. This section cut through the brain stem 2 mm. in front of the anterior colliculi, and through the posterior part of the corpus mammillare. Most of the diencephalon was thus removed.
7:45 p.m.	Rectal temp. 33.4°C.—room temp. 22°C.
9:45 p.m.	Rectal temp. 31.4°C.—room temp. 22.6°C.—animal placed in front of open window.
10:15 p.m.	Rectal temp. 30.4°C.—no sign of shivering—corneal reflexes, knee jerks active.
10:30 p.m.	Rectal temp. 29.0°C.—shivering occurs occasionally in the hind limbs—it lasts for a few seconds on each occasion, and then reappears when the animal is placed on its back.

Shivering after removal of midbrain and pons. There are five positive results in this series, the transection levels being as follows:

Rabbit 38—through the middle of the anterior colliculi and the posterior border of the corpus mammillare (fig. 2).

Rabbit 36—through the posterior border of the anterior colliculi and the upper border of the pons.

Rabbit 40—through the anterior portion of the posterior colliculi, and the anterior border of the corpora trapezoidea (fig. 3).

Rabbit 42—immediately behind the posterior colliculi, and the anterior border of the corpora trapezoidea (fig. 4).

Rabbit 45—same as in 42.

Two of the protocols are given below.

February 8, 1929. Rabbit 38. Weight, 1.9 kgm.

10:30 a.m. Given 0.3 cc. amytal per kgm. intravenously—both common carotid arteries ligated—skull trephined over occipital lobes.
11:30 a.m. to 12:00 m. Shivering induced by placing the animal in front of open window.
12:00 m. Window closed—rectal temp. 36.7°C.
12:05 p.m. Brain transected—shivering stopped on completion. This cut (fig. 2) passed from the level of the middle of the anterior colliculi dorsally, to the posterior margin of the corpus mammillare ventrally.
12:10 p.m. Marked rigidity is present in both left limbs, particularly in the left hind limb—there is very little increased tonus in the right limbs.
12:15 p.m. Shivering occurs in the fore limbs, and occasionally in the left hind limb——it is not vigorous.
2:00 p.m. Rectal temp. 34.7°C.—there is no spontaneous shivering noticeable now—vibrissa and corneal reflexes absent; knee jerks hyperactive—animal placed in front of open window.
3:00 p.m. Rectal temp. 33°C.
4:00 p.m. Shivering is quite marked.
5:00 p.m. Animal is shivering vigorously, and showing progression movements.

March 8, 1929. Rabbit 45. Weight, 1.8 kgm.

11:00 a.m. Amytal anesthesia—both carotid arteries ligated—tracheotomy—skull over cerebellum removed.
3:00 p.m. Brain stem transected. This cut (fig. 4) passed dorsally behind the posterior colliculi, ventrally through the upper border of the corpora trapezoidea; marked rigidity came on almost immediately.
4:20 p.m. Rectal temp. 39.2°C.
4:30 p.m. Tremors of the fore limbs occur when the animal is placed on its back—rigidity is strongly maintained.
4:35 p.m. Placed in front of open window—external temp. 1°C.
4:37 p.m. Spontaneous shivering occurs in the limbs when they are forcibly flexed—there is very little shivering in the rigidly extended limb.
5:00 p.m. Rectal temp. 35.8°C.—there is now moderate spontaneous shivering.
5:45 p.m. Rectal temp. 34°C.—moderate shivering still present—window closed—room temp. 20°C.
6:00 p.m. Rectal temp. 34.6°C.—moderate shivering.
6:20 p.m. Rectal temp. 35.8°C.—moderate shivering.
7:00 p.m. Rectal temp. 37.3°C.—moderate shivering.
8:00 p.m. Rectal temp. 39.6°C.—moderate shivering.
9:10 p.m. Animal killed, brain removed.

In the four last mentioned experiments the lines of transection passed obliquely through part of the rhombencephalon without prevention of shivering. Observations on animals after sections at lower levels follow.

Shivering in medulla animals. A. *Experiments on the upper medulla.* There are 7 experimental results, 4 obtained in animals operated under amytal anesthesia, 3 under ether. The transection levels were as follows:

i. Under amytal. Rabbit 43—dorsally, 3 mm. behind the posterior colliculi, ventrally, middle of the corpora trapezoidea (fig. 5).
Rabbit 44—same as 43 (fig. 6).
Rabbit 47—dorsally, upper part of the corpora restiformia—ventrally, middle of corpora trapezoidea, through the 8th nerve (fig. 7).
Rabbit 50—dorsally, upper part of corpora restiformia—ventrally, lower border of corpora trapezoidea, below the 8th nerve.

ii. Under ether. Rabbit 51—dorsally, 5 mm. above the calamus scriptorius—ventrally, lower border of corpora trapezoidea (fig. 8).
Rabbit 52—same as 51 (fig. 9).
Rabbit 54—dorsally, 2 mm. above the calamus scriptorius—ventrally, immediately behind the 8th nerve (fig. 10).

Typical shivering was observed in all of these animals. Some of the abridged protocols are given here.

Rabbit 43—10:00 a.m. Amytal anesthesia—carotid arteries ligated—cerebellum exposed by mid-line trephine.

4:45 p.m. Moderate shivering induced by exposure for short periods to cold draft. Transection carried through middle cerebellar lobe to base of brain (fig. 5)—there was a gush of blood, finally arrested by vertebral compression—shivering not affected by the cut.
4:50 p.m. Rectal temp. 37.8°C.—animal shivering strongly.
5:30 p.m. Rigidity present in hind limbs.
7:00 p.m. Rigidity present in all limbs and neck muscles.
7:40 p.m. Rectal temp. 37°C.—the shivering is diminishing—placed in front of open window: external temp. 6°C.
8:05 p.m. Rectal temp. 34.8°C.—moderate shivering again present.
8:35 p.m. Rectal temp. 32.2°C.—animal shivering strongly—window closed; room temp. 22°C.
9:20 p.m. Shivering decreasing—marked rigidity present.
10:20 p.m. Rectal temp. 33.3°C.—moderate shivering.
11:00 p.m. Rectal temp. 34°C.—room temp. 21°C.
12:00 p.m. Rectal temp. 35°C.—animal killed, brain removed.

Rabbit 50—10:20 a.m. Amytal anesthesia—carotid arteries ligated—tracheotomy—skull trephined over middle cerebellar lobe.

12:15 p.m. Shivering induced by exposure to cold.
1:50 p.m. Rectal temp. 37.6°C.—shivering strongly—artificial respiration started —incision carried through cerebellum to base of brain—very little hemorrhage occurred. This cut went from the upper part of the

TABLE 1

EXPERIMENT NUMBER	LEVEL OF TRANSECTION	TIME BETWEEN TRANSECTION AND RESUMPTION OR ONSET OF SHIVERING	MAXIMUM GAIN IN BODY TEMPERATURE WITH SHIVERING	APPROXIMATE TEMPERATURE OF ENVIRONMENT
				°C.
39	Hemispheres removed	Shivering not stopped	1.8°C. in 50 minutes	23
27 (transection 2)	Anterior thalamus; behind optic chiasma	5 minutes	Cooled throughout experiment	
31 (transection 3)	Anterior third thalamus; middle of corpus mammillare	1 hour	Not measured	
27 (transection 3)	Front of anterior colliculi; posterior border of corpus mammillare	2 hours, 50 minutes		
30	Front of anterior colliculi; posterior border of corpus mammillare	7 to 8 hours	Loss of 4.7°C.	23
38	Middle of anterior colliculi; posterior border of corpus mammillare	4 hours	Not measured	
36	Posterior border of anterior colliculi; anterior border of pons	ca. 3 hours		
40	Anterior border of posterior colliculi; anterior border of corpora trapezoidea	ca. 4 hours	Loss of 1.5°C.	23
42	Behind posterior colliculi; anterior border of corpora trapezoidea	1-1½ hours	2.9°C. in 3 hours, 10 minutes	24
45	Behind posterior colliculi; anterior border of corpora trapezoidea	1-5 minutes	5.6°C. in 2 hours, 15 minutes	20
43	3 mm. behind posterior colliculi; middle of corpora trapezoidea	1-5 minutes	3.7°C. in 3 hours, 5 minutes	21
44	3 mm. behind posterior colliculi; middle of corpora trapezoidea	55 minutes	1.4°C. in 3 hours, 50 minutes	24
47	Upper part of corpora restiformia; middle of corpora trapezoidea	1 hour, 10 minutes	2.2°C. in 3 hours, 20 minutes	24
50	Upper part of corpora restiformia; lower border of corpora trapezoidea	2 hours, 10 minutes	2.4°C. in 3 hours, 25 minutes	25.5
51	5 mm. above calamus scriptorius; lower border of corpora trapezoidea	25 minutes	3.7°C. in 1 hour, 30 minutes	26.6
52	5 mm. above calamus scriptorius; lower border of corpora trapezoidea	25 minutes	2.8°C. in 3 hours, 32 minutes	22.6-26.3
54	2 mm. above calamus scriptorius; lower border of corpora trapezoidea	3 hours, 50 minutes	0.7°C. in 40 minutes	23
53	Through calamus scriptorius	?	Loss	23
55	2 mm. below calamus scriptorius	2 hours, 50 minutes	Loss	24
56	2 mm. below calamus scriptorius	?	Loss	23
57	Cerebellum removed	Shivering not stopped	2.7°C. in 1 hour, 5 minutes	23

corpora restiformia to the lower border of the corpora trapezoidea, below the 8th nerve.

1:55 p.m. Fine tremors of the fore limbs can be observed, but no shivering.

2:30 p.m. Spontaneous respiration reëstablished—heart action weak, and varies from moment to moment.

3:00 p.m. Heart action is now regular, at about 240 beats per minute.

3:10 p.m. Placed in front of open window—outside temp. 18°-20°C.—rectal temp. 37.2°C.

4:00 p.m. Rectal temp. 36.3°C.—shivering is present in both fore limbs.

5:10 p.m. Rectal temp. 33.3°C.—shivering also occurs from time to time in the hind limbs.

6:35 p.m. Rectal temp. 30.6°C.—moderate general shivering present—window closed; room temp. 25.5°C.

9:00 p.m. Rectal temp. 32°C.—moderate shivering—room temp. 25.2°C.

10:00 p.m. Rectal temp. 33°C.

Rabbit 54—3:00 p.m. Ether anesthesia—carotid arteries ligated—tracheotomy—artificial respiration at rate of 32 per minute—atlanto-occipital membrane exposed and divided.

3:40 p.m. Medulla sectioned, with care not to injure the cerebellum—practically no bleeding occurred—the incision passed through the medulla 2 mm. above the calamus scriptorius (fig. 10).

4:00 p.m. Artificial respiration still required—condition of animal fair—tendon reflexes hyperactive.

4:15 p.m. Rectal temp. 37.3°C.—room temp. 22°C.—there are fine visible tremors of the fore limbs—windows in the room opened.

5:30 to 6:00 p.m. Clonic twitchings or spasms occur occasionally in the hind limbs, associated with coarse tremors—rectal temp. 34.2°C.—room temp. 17.6°C.

7:30 p.m. Rectal temp. 29.5°C.—room temp. 16°C.—there is now spontaneous shivering in all limbs, as well as in the trunk muscles.

8:30 p.m. Rectal temp. 30°C.—room temp. 22°C.

10:00 p.m. Rectal temp. 30°C.—room temp. 23°C.—shivering much decreased—window again opened.

10:30 p.m. Rectal temp. 29.4°C.—room temp. 19°C.—moderate shivering again present.

11:10 p.m. Rectal temp. 30.1°C.—room temp. 21°C.

Records of shivering movements from experiments 50, 51, 52, 54 are shown in figure 12. They were obtained by connecting one of the shivering limbs to a tambour, which moved a light lever over a kymograph. Variations in the position of the animal as well as in the amount of movement in any one limb from time to time make comparison of these records impossible. The influence of cold stimulation upon the intensity of the shivering is shown in figure 12, d.

The shivering after ablation of the midbrain and of the more posterior parts of the brain stem was, as the protocols indicate, much more pronounced than that seen in the diencephalon animals. The difference in extent of reaction to cold may be accounted for by the relatively better

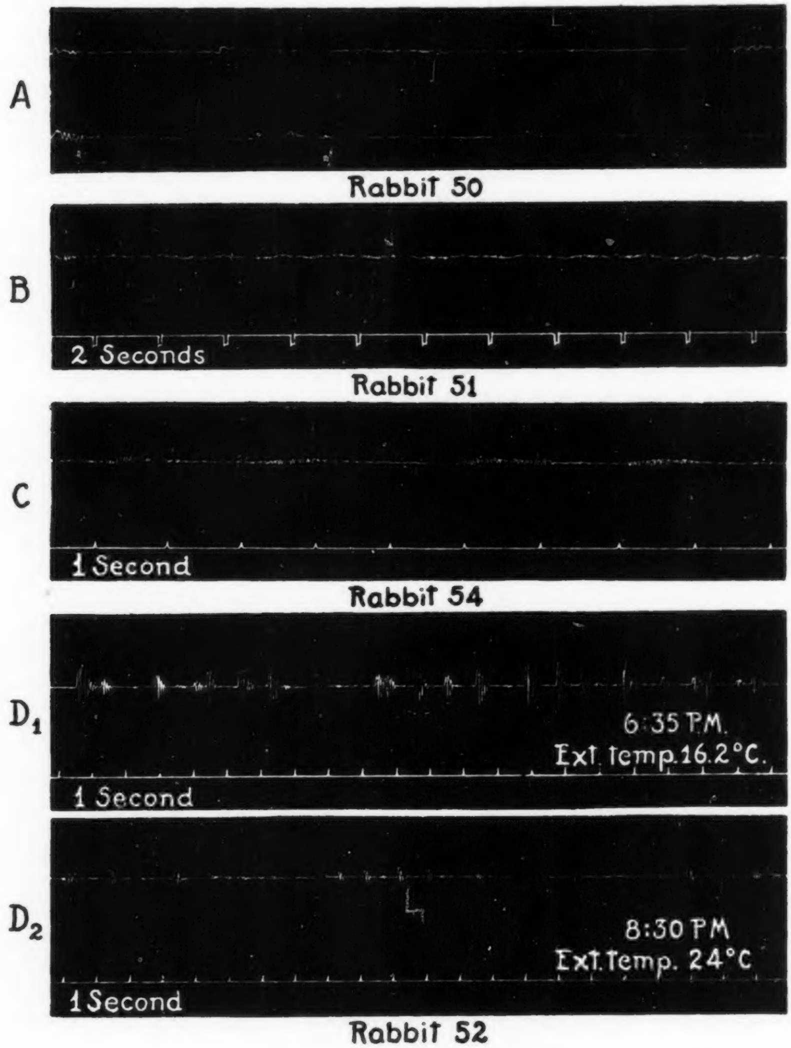

Fig. 12. Shivering after transections through the medulla oblongata

condition of the former animals. An incision through the diencephalon was always followed by much hemorrhage; in most cases the bleeding was progressive and impossible to arrest entirely. Practically always a large mass of brain tissue was raised up by the blood gathering within the cranial cavity and was pushed through the trephine hole. Owing to the progressive loss of blood, the abnormal intracranial pressure, and the continuous destruction of brain tissue, the condition of these animals became worse as time went on. In the case of the medulla animals the reverse was found to hold good. Generally, there was more immediate "shock" following the cut, so that many animals died within a short time of cardiac or respiratory failure. But in the surviving animals the shock passed off with remarkable rapidity; the reflexes soon returned; there was practically no appreciable hemorrhage, and comparatively little trauma. Often it was possible, by means of the blunt instrument described, to cut through the medulla without severing the basilar artery, so that when the brain was removed at the end of the experiment the sectioned portions were held together on the ventral aspect by this vessel. When this artery was damaged, the bleeding could be controlled quite readily by intermittent compressions of the vertebrals. Several hours after the operation such an animal was always found to be in better condition than an animal with the diencephalon or midbrain transected.

In none of the experiments was shivering ever observed at a time when the more common spinal reflexes were absent. This is in accordance with the observations of Pembrey (1901), of Britton (1922), and of Sherrington (1924). A high reflex excitability of the spinal cord seems to be prerequisite for the demonstration of the shivering reflex.

The transection level in rabbit 54 is the lowest at which typical shivering movements appeared; it shows that this reflex is still present after all of the brain stem, up to 2 mm. in front of the calamus scriptorius, has been removed.

B. *Experiments on the lower medulla.* There were only three results here that may be regarded as significant, out of a comparatively large number of attempts. The difficulties attending operative procedures in the vicinity of the *noeud vitale* were found to be almost insurmountable. Over 20 rabbits survived the operation for more than one hour. Artificial respiration was required throughout; in most cases the heart action was extremely weak and apt to stop at any moment after the incision. Ten such animals were exposed to cold for a time, and 3 of these showed undoubted muscular response—rabbits 53, 55, 56.

The muscular response to cooling in these preparations consisted of two components; one, a coarse twitching or spasm, affecting the trunk and limbs; the other, an irregular, fine tremor, usually but not always bilateral. In the case of rabbit 53, the twitching was elicited only after exposure to

an external temperature of 9°C. for one hour; it tended to disappear when the animal was not actively cooled. The medulla of this animal was divided at the level of the calamus scriptorius.

In rabbit 55 the transection passed 2 mm. below the calamus (fig. 11). Two hours and fifty minutes after the incision, when the rectal temperature had fallen to 30°C. as the result of active cooling, slight tremors were felt in all the limbs. These were associated with the type of twitching described. At times an irregular, incoördinated shivering was seen in various parts of the body. The muscular activity was apparently local and independent of that in any other part of the body, e.g., the contralateral limb. This is a very different picture from that of typical thermal shivering. But the fact that it was brought on by cooling, and decreased when the animal was placed in warmer surroundings indicates some relation to true shivering.

The cut in rabbit 56 was in the same region. This animal, after exposure to a cold draft for fifty minutes, showed the gross twitching described. Nothing analogous to typical shivering was observed in this preparation, even when the rectal temperature had been lowered to 27.3°C.

Shivering after removal of the cerebellum. There was the possibility that the modified "shivering" observed in the last mentioned experiments was due to severance of connection between the cerebellum and the portion of the brain stem remaining. To settle this point attempts were made to remove the cerebellum and leave the brain stem intact. The extirpation was successfully accomplished in rabbit 57. Typical, vigorous shivering, entirely like that of the normal rabbit, supervened after the animal had been left for a time in a cool room. The shivering increased greatly when the rabbit was placed in a cold chamber (temp. 4°C.). It is evident that the cerebellum plays no essential rôle in thermal shivering.

One must consider as a possible cause of the muscular reaction observed in these experiments some dynamic or irritative factor, brought into play by the brain injury itself. Indeed, in experiments of this type, such a possibility can never be excluded. But the following facts argue strongly in favor of its being primarily a response to cooling: 1, a certain time always elapsed between the brain section and the onset of shivering (cf. table 1); 2, in nearly every case the shivering supervened only after prolonged and severe cold stimulation; 3, shivering tended to wear off as the temperature of either the animal or the environment was raised; 4, there was most shivering in the cases where the injury to the brain stem was least; 5, the shivering could be greatly increased by afferent stimulation, as in the normal rabbit under light anesthesia; 6, the general picture resembled that of normal shivering very closely.

These experiments show that as the brain stem in the rabbit is sectioned progressively caudad, good shivering capacity is maintained as far down as the lower part of the medulla oblongata (cf. expt. 54). At about the level of the calamus scriptorius typical shivering is replaced by a form of

muscular activity less integrated, less generalized, and less intense. The transition between these forms of reaction is not a sharp one; both are certainly evidence of muscular response to cold. It follows that a central control of shivering in the rabbit must be situated in the lower medulla or upper cervical cord.

The fact that in most instances shivering stopped immediately after the transection and reappeared only after further cooling indicates that the threshold for shivering was abnormally high in these preparations. Under the conditions it was not possible to ascertain whether the raised threshold was due to the effect of the trauma, or to the severance of connections with some of the upper regions of the brain stem. It is pertinent in this regard to consider that removal of the cerebral hemispheres and of the cerebellum can be accomplished without affecting shivering to any extent (cf. expts. 39 and 57). The motor side of the reflex was influenced very little by transection of the brain stem as low as 2 mm. above the calamus scriptorius.

Observations on decerebrate rigidity. In this rather long series of experiments decerebrate rigidity was first observed in rabbit 38, when the transection passed from the middle of the anterior colliculi to behind the corpus mammillare. The fact that rigidity does not necessarily supervene until most of the red nucleus has been ablated has been established by Rademaker (1926). As the brain stem was sectioned progressively backward in successive experiments, marked extensor tone was always present until a level was reached well down in the medulla. The lowest section followed by rigidity was in rabbit 43; here the cut passed from a point 3 mm. behind the posterior colliculi dorsally to the middle of the corpora trapezoidea ventrally. In this animal the rigidity was strongly maintained for over six hours, at the end of which time the rabbit was killed. In rabbit 44, in which the brain stem was sectioned at the same level, no exaggerated muscle tone was evident. In the subsequent experiments the extensor tone was, so far as could be judged by mere inspection, in no way increased. If anything, the attitude of the animals tended toward hyperflexion; in the hind limbs the knees were usually drawn up towards the body, while in the fore limbs the elbows, wrists, and even phalanges were strongly flexed.

Rademaker (1926) has pointed out that in the rabbit decerebrate rigidity is nearly always more marked in the hind limbs than in the fore limbs. The present observations also showed this to be the case; further, rigidity was occasionally present in the hind limbs some time before it became manifest in the fore limbs.

In experiments 36, 38, 40, 42, 45, 43, shivering occurred during marked decerebrate rigidity. The question of a possible relationship between these phenomena is an interesting one. It is noteworthy that, like shivering, decerebrate rigidity becomes less intense as the medulla oblongata is

sectioned progressively backward. Thus Magnus states: "Durchtrennt man bei decerebrierten Katzen nach vorheriger Kleinhirnextirpation die Medulla oblongata durch eine Reihe sich von vorne nach hinten folgender Frontalschnitte, so nimmt allmählich die Enthirnungstarre an Intensität ab." A comparison of the mode of innervation of both types of reaction would be of interest.

The central control of heat regulation. After shivering had been induced by exposure to external temperatures varying from 25°C. to −7°C.—with consequent lowering of the body temperature—the rabbits were left for a time in an ordinary room (temp. between 20°C. and 26°C.). Many of them were able, as the result of shivering, to raise their body temperature; the extent of the increases observed in the different experiments is shown in table 1.

It will be noted that rabbit 54, after transection 2 mm. above the calamus scriptorius, gained 0.7°C. in 40 minutes, while rabbits 53, 55, 56, in which the medulla was divided at or below the calamus, all showed a loss in body temperature under not very dissimilar conditions.

It is, of course, not altogether impossible that the temperature increases noted were due not to shivering, but to some irritation, analogous to heat puncture, in the brain stem. Against this are the following considerations: 1, previous to the onset of shivering, most of the preparations showed a continuous fall in body temperature; 2, the gain was roughly proportional to the amount of shivering observed; 3, it was not observed in all of the animals; 4, in the large number of negative shivering experiments no gain in body temperature exceeding 1°C. in a few hours was ever observed; the almost universal result in the absence of shivering was an uninterrupted cooling of the animal at a rate determined by the temperature of the surroundings.

The significance of these observations from the viewpoint of heat regulation can be considered only after we have defined the rôle played by shivering therein. Considerable light has been thrown on this subject by the recent work of Cannon, Querido, Britton and Bright (1927). As is well known, an animal subjected to cooling (beyond the range of physical regulation) must meet an impending heat deficit by increase in metabolism. This increase may be accomplished in two ways—by augmented discharge from the adrenal medulla, and by shivering. The activation of the sympathico-adrenal mechanism is the first line of defense; "hair or feathers are lifted till a thicker layer of poorly conducting air is enclosed about the body, peripheral blood vessels are constricted so that the escape of heat by radiation and conduction is diminished, easily combustible sugar is liberated into the blood stream, and adrenin which accelerates combustion is put forth in greater amounts." By this means an increase in metabolic rate up to 30 per cent may be evoked to counteract the effects of cooling. If this first line of defense proves inadequate, and the temperature tends

to drop in spite of it, there is a second line in the control of muscular action by the nervous system—shivering is evoked. With the onset of shivering a much greater increase in heat production occurs.

The relation of shivering to heat regulation, therefore, is clear to this extent—that insofar as active response to cold is a function of the heat-regulating mechanism, shivering must be considered as an integral part of this mechanism.

The central control of heat regulation has interested investigators for many years. The preponderance of evidence adduced from ablation and stimulation experiments points to a diencephalic centre. Thus, all observers agree that, as the brain is sectioned from before backward, the first obvious breakdown of the temperature-regulating mechanism occurs when the diencephalon is injured (Isenschmid and Krehl, 1912; Isenschmid and Schnitzler, 1914; Rogers, 1919). The results obtained from stimulation of this and other regions in the brain stem are not fully convincing; Bruman (1929) who has recently repeated some of this work regards the issue as still unsettled (cf. Kayser, 1929).

The fact that shivering could be induced in the animals described in this communication indicates that functional ablation of the diencephalon does not destroy all vestiges of temperature control. Indeed, not only shivering, but several of the other factors that play a rôle in the response of an animal to temperature may be elicited, as isolated responses, from levels of the central nervous system far below the diencephalon. This has long been known as regards vaso-motor and respiratory activity, while Cannon and Rapport (1921) have demonstrated that reflex adrenin secretion may be stimulated through a centre below the midbrain. Through the action of adrenin, pilo-motor and vascular reactions, as well as an increase in heat production, may be effected. It has not been shown that, in the absence of the diencephalon, any of these reactions can be evoked in response to changes in temperature, but the possibility remains.

There is no reason to suppose, however, that any one of these items, including shivering, is alone capable of keeping constant the body temperature of a homeothermic animal in the face of wide fluctuations in the temperature of the environment. In fact, it is probable that complete thermotaxis is possible only when most, if not all, of these factors can be effectively brought into play. Thus Martin (1903) states that lower mammals, which lack some of the means of thermal adjustment found in the more highly developed mammals and birds, regulate their temperature only imperfectly. In the diencephalon most of these items are represented, and the importance of this part of the brain stem in the maintenance of a constant temperature is, apparently, due to its function of coördinating the various responses of the organism to changes in the environmental temperature.

It is interesting that the reaction of an animal during physiological rage is controlled by the diencephalon in a similar manner. As Bard (1928) has pointed out, many of the elements of behavior that, together, constitute the typical rage reaction, may be induced as isolated responses in the midbrain animal, and even in the bulbo-spinal and spinal preparation; but only in the presence of the diencephalon is the picture typical of the true pseudaffective state realized. When this part of the brain stem is functionally absent, the character of the rage reaction is markedly altered as regards intensity and extent. In both instances the diencephalon functions not as an independent, autonomous centre, in the strict sense of the term, but as a coördinating centre, where the separate responses are welded together to form the generalized, typical, and hence effective, response.

SUMMARY

In a series of experiments on the localization of the central control of shivering in the rabbit, the brain stem was transected at various levels, and the shivering capacity tested shortly thereafter. Shivering still occurred after complete transection about 2 mm. above the calamus scriptorius, although there is evidence that after transection through or below the diencephalon, the threshold for this reflex is raised. At about the level of the calamus typical shivering was replaced by a form of muscular response less integrated, less generalized, and less intense, in the form of clonic spasms and incoördinated tremors. It would seem that the intensity of muscular response to cooling is diminished gradually as the medulla oblongata is progressively sectioned; there is no abrupt disappearance of shivering. Inasmuch as decerebrate rigidity is similarly affected by successive cuts through the medulla, the possibility of a relation between these phenomena is of interest.

Gains in body temperature due to shivering occurred in many of the animals, indicating that, with most of the brain stem functionally removed, some vestige of the heat regulating mechanism was still present. The bearing of this on the question of a diencephalic centre for heat regulation is discussed.

I wish to express my thanks to Prof. W. B. Cannon, who suggested this problem, for his helpful criticism and advice in the interpretation of some of the results.

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THE EFFECT OF EMOTION ON THE BLOOD-PLATELET COUNT

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The rôle of the spleen as a reservoir for erythrocytes was first suggested by Gray in 1854 and is now generally accepted, due to the work of Barcroft and his co-workers (1923–1927) and to others who have shown that many circumstances affecting the organism—CO poisoning, muscular exercise, injections of adrenin and pituitrin, etc.—cause the spleen to contract, thereby discharging into the general circulation an extra supply of red blood corpuscles which have been held there. The effect of emotional excitement in raising the erythrocyte count has been pointed out by Ferrari (1897) and by Lamson (1915–1920), and it has been explained by Izquierdo and Cannon (1928) as the result of splenic contraction which is known to be effected by emotional states.

Binet and Kaplan (1927–1928) have been led to consider the spleen as a reservoir for platelets as well as for erythrocytes. Working with dogs, they found that in acute asphyxia there is a noteworthy increase in platelets, often as many as 200,000 per cubic millimeter, an increase which does not take place in splenectomized animals. They have also shown that if splenic contraction is brought about in a dog by the intravenous injection of adrenin or ephedrine, there is a great increase in the number of platelets, which does not occur in splenectomized dogs or in dogs previously injected with yohimbine, under which condition the spleen loses its ability to contract.

The present work was undertaken to learn whether emotional excitement in a normal animal would cause an increase in the number of platelets, since emotion, by stimulating the sympathetic system, would cause the spleen to contract just as adrenin does. Furthermore, if the effect should occur in consequence of the action of the sympathetic system on the spleen, then in those animals in which the sympathetic system has been removed, and in which the spleen has been either removed or denervated, there should be no appreciable increase.

Method. Normal cats, cats in which the heart had been denervated and from which the stellate ganglia and the thoracic and abdominal sympathetic strands had been removed (Cannon, Lewis and Britton, 1926), and splenectomized cats were used.

The blood was taken from a slight cut in the ear directly into a diluting pipette. The animals were held as quietly as possible, though undoubtedly the occasional high initial count can be attributed to slight excitement noticed in some instances. The diluting fluid was 0.7 per cent NaCl and 0.3 per cent Na citrate. After the blood was diluted, the pipette was shaken vigorously for two minutes; the counting chamber (Leitz) was then charged and allowed to stand for ten minutes before the count was made. As a rule, 200 squares were counted, a check count made of each slide, and the two averaged. After the control blood sample was taken, the animal was tied to a board and excited by this restraint for a period of three minutes. Immediately following this, a second sample was taken and further samples were taken at intervals of 15, 30, and 60 minutes following the period of excitement.

TABLE 1

Effect of three minutes of excitement on the platelet count in normal cats

DATE (1929)	CAT NUMBER	BEFORE EXCITEMENT	IMMEDIATELY AFTER	15 MINUTES AFTER	30 MINUTES AFTER	60 MINUTES AFTER	PERCENTAGE CHANGE
February 27	1	176,000	336,000	140,000		240,000	+90
March 1	2	448,000	752,000		192,000	300,000	+67
March 2	3	480,000	608,000		184,000	200,000	+27
March 4	2	400,000	604,000		304,000	304,000	+26
March 5	3	200,000	448,000		248,000		+80
March 7	4	416,000	688,000		616,000	472,000	+65
March 8	1	164,000	264,000		172,000	216,000	+60
March 14	5	392,000	600,000			444,000	+53
March 18	6	500,000	476,000		340,000		−4
March 21	7	276,000	417,000			328,000	+50
Average		345,300	508,000		312,500	293,306	+51.4

Effect of excitement on platelet count in normal cats. Table 1 shows the effect of three minutes' excitement on the platelet count of normal cats. It is clear from these data that in every animal, with the exception of cat 6, there is a sudden and striking increase in the number of platelets immediately after the excitement, an average increase of over 50 per cent. This change is somewhat variable, ranging from a decrease of 4 per cent in the one case mentioned, to an increase of 90 per cent. On the whole, the higher the initial count, the less the change after excitement. In cat 6, which showed the highest initial count, there was, after the excitement, a slight decrease, negligible probably because close to the range of accuracy of the method. This cat showed obvious signs of agitation, such as switching of the tail, dilated pupils, etc., before the control sample was taken; the condition may account for the high initial count and for the absence of increase after the excitement.

It will be noted that in some cases the platelet count 30 minutes after the period of excitement was considerably lower than the initial count. Where this was the case, the count rose during the next 30 minutes to approach more nearly the initial level.

TABLE 2

Effect of three minutes of excitement on the platelet count in sympathectomized cats

DATE (1929)	CAT NUMBER	BEFORE EXCITEMENT	IMMEDIATELY AFTER	30 MINUTES AFTER	60 MINUTES AFTER	PERCENTAGE CHANGE
March 25	408	460,000	344,000	476,000	292,000	−25
March 26	384	428,000	412,000	540,000	400,000	−3.7
March 27	384	488,000	576,000	344,000	396,000	+17.8
March 28	408	200,000	244,000	304,000	300,000	+22.0
March 29	408	276,000	258,000		496,000	−6.0
March 29	408	304,000	340,000	352,000	216,000	+11.0
April 2	403	580,000	448,000	768,000	429,000	−22.0
April 3	403	620,000	572,000	488,000	464,000	−7.9
April 3	403	458,400	460,000	448,000	476,000	−0.3
April 4	403	632,000	611,600	400,000	624,000	−3.2
April 5	384	316,000	416,000	340,000	412,000	+31.0
April 5	384	660,000	504,000	872,000	416,000	−8.4
Average		451,800	432,100	485,100	410,400	−4.3

TABLE 3

Effect of three minutes of excitement on the platelet count in splenectomized cats

DATE (1929)	CAT NUMBER	BEFORE EXCITEMENT	IMMEDIATELY AFTER	30 MINUTES AFTER	60 MINUTES AFTER	PERCENTAGE CHANGE
May 10	452	648,000	560,000	560,000	584,000	−13.0
May 10	453	520,000	416,000	340,000	456,000	−20.0
May 11	451	512,000	496,000	496,000	406,000	−3.0
June 5	450	632,000	584,000	576,000	536,000	−8.0
June 6	451	424,000	376,000	384,000	296,000	−11.0
June 6	452	520,000	427,000	440,000	400,000	−19.0
June 7	450	416,000	304,000	288,000	285,000	−26.0
Average		524,000	451,000	441,000	424,000	−14.3

Effect of excitement on platelet count of sympathectomized cats. Table 2 shows the effect produced by three minutes of restraint in sympathectomized cats. The average change, following the period of excitement, was a decrease of 4.3 per cent. In 4 of the 12 experiments there was an increase after the excitement, though in only one case was it as great as in the normal cats.

Effect of excitement on the platelet count of splenectomized cats. Table 3 shows the effect of three minutes' excitement on cats whose spleens had been previously removed. These cats responded in the same manner as the sympathectomized cats, though with more regularity. In all cases there was a decrease in the number of platelets after the period of excitement, the average decrease being 14.3 per cent.

SUMMARY

1. Excitement for three minutes causes a sudden increase in the number of blood platelets in the circulating blood of normal cats.

2. Sympathectomized cats show, on the average, a slight decrease in the platelet count after the same period of emotional excitement.

3. In splenectomized cats there is regularly a decided decrease in the number of blood platelets after excitement for three minutes.

I wish to express my obligation to Dr. W. B. Cannon for advice and encouragement given during the course of this investigation.

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THE ENERGY METABOLISM OF PREGNANT RABBITS

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Practically all studies of the metabolism of pregnancy have consisted in determining how the metabolic processes of the mother, otherwise in a state of equilibrium, are modified by the pregnant state. So far as energy metabolism is concerned, most workers have attempted to find if there is an active increase during pregnancy. Those who have demonstrated such an increase have further been concerned with studying its relation first, to the weight of the mother, second, to the surface area of the mother, and third, to the combined surface area of mother and fetus.

The literature on this subject may be found in the articles by Boothby and Sandiford (1924), and Harding (1925), and in Lusk (1928). Only a few of the papers, particularly those having a bearing on the problems already mentioned, need be discussed here. Magnus-Levy (1904) found an increase in oxygen consumption per unit of weight in one human as early as the third month of pregnancy. Zuntz (1910) reported two cases which did not show any increase in oxygen consumption, or if there was any it was only a small amount shortly before parturition. In dogs Murlin (1910) found an increased heat production which at the culmination of pregnancy was proportional to the weight of the offspring at birth. This extra energy was very nearly equal to the energy requirements of the newborn when calculated according to the law of skin area. In three human pregnancies Carpenter and Murlin (1911) noted that there was no deflection of the curve of total energy production of mother and child at birth. They believed that the heat production per unit of surface area was greater in pregnant women, delivered women, and newborn young than it was in women at sexual rest. It should be noted that if the higher normals of Benedict and Emmes (1915) are used these differences are not so apparent.

Although Baer (1921) and Cornell (1923) found metabolic increases in pregnant women and a decline after delivery, their work has been discounted by reviewers because many pathological cases were included. In a careful study of one patient from the 4th month to 11 days before delivery, Root and Root (1923) have reported an increase of 23 per cent in total calories per day and an increase of 7.6 per cent in calories per kilo.

These increases the authors believed to be out of all proportion to those shown by standard prediction tables for normal women who had increased equally in weight. Rowe, Alcott and Mortimer (1925) also showed in a large series of cases that of the increased heat production in pregnant women, which amounted to about 1 per cent per week, only 4 per cent was referable to increased weight. The linear character of their curves early in pregnancy seemed to indicate that the mere amount of fetal protoplasm could not condition the increased energy requirements.

A remarkably complete record of basal metabolism in one subject before, during and after pregnancy has been published by Sandiford and Wheeler (1924). These workers found in agreement with others that the total calories per hour, the calories per square meter per hour and the calories per kilo per hour were all increased during the latter part of pregnancy. If however the weight of the mother and fetus were considered separately, and the surface area for each determined and combined, the rate in calories per square meter of surface for the maternal organism seemed to be unchanged throughout pregnancy. Sandiford and Wheeler furthermore showed that if the data of other observers were treated in the same way, the results were not inconsistent with the conclusion that the energy production of a unit mass of the mother's protoplasmic tissue remained unchanged throughout the course of pregnancy.

In the work reported here rabbits have been used. The pregnant period in this animal is relatively short and considerable material is therefore easily available. The heat production before, during, and after pregnancy has been determined, as well as the metabolism of the new born. Attempts have been made to correlate the heat production with weight and surface area.

Apparatus and method. The animals used were female rabbits weighing from 2 to 4 kilograms. In all cases they were kept off feed for 12 to 24 hours preceding the tests.

The apparatus used was a modified Haldane open circuit type, similar to that described by Marine (1922). The rate of air flow was about 5 liters per minute. Repeated tests demonstrated the apparatus to be air-tight and numerous alcohol tests showed the efficiency of the carbon dioxide and water absorbers to be within 2 per cent.

A two hour period of observation was the general rule. This long period minimized to a great degree any effects occasioned by the uncontrollable activity of the animal. As a matter of fact after the initial test the animals seldom moved during the succeeding periods. A roughened board in the bottom of the chamber seemed to add greatly to their comfort and quietness. The animal chamber was made of metal with a glass door. Observations showed that the temperature in the box did not vary sufficiently to modify the experiments.

The weighings were made rapidly on a Sauter balance, the accuracy of which was plus or minus 10 mgm. per 10 kilograms. The heat production was calculated in the usual way which is detailed in Marine's publication.

Metabolism of normal rabbits. In order to have a basis of comparison for the experimental groups it was necessary to determine the average heat production of normal rabbits. Marine (1922) found that the normal range was between 2.40 and 2.50 calories per kilogram per hour. More recently Webster, Clawson and Chesney (1928) have reported that the average heat production in rabbits was 2.64 calories per kilogram per hour. We have made 76 determinations on the 36 rabbits subsequently used in our experiments. In calories per kilo per hour the minimal figure obtained was 1.92, the maximal was 3.25 and the average was 2.61. Sixty-seven per cent of all the figures fell between 2.30 and 2.90. These figures compare favorably both in range and average with the results of previous workers, and it may safely be concluded that the basal metabolism of normal female rabbits is not far from 2.61 calories per kilo per hour.

The extent to which variations in weight affect the calories per kilo is a question that must be considered in determining the significance of an average figure. If the heat production in rabbits is remarkably constant per unit of surface area, as we shall show presently, it is obvious that the calories per kilo must fall as the weight rises. Webster, Clawson and Chesney (1928) felt this divergence was so slight within the weight range of adult rabbits that surface area might be ignored and all observations be expressed on the basis of weight alone. Our figures do not wholly justify this conclusion, but they do show that the average of calories per kilo per hour is quite constant for rabbits weighing 1900 to 3000 grams. The group weighing 1900 to 2400 grams averaged 2.70; the group weighing 2500 to 3000 grams averaged 2.63; the small group weighing over 3000 averaged 2.24. The influence of weight may then be noticeable in the larger animals. It must also become noticeable in small animals but our data do not cover this point.

In recent years there has been pretty general agreement that the resting metabolism of animals is proportional to their surface area. In attempting to state the heat production of rabbits in calories per square meter certain difficulties at once arise. There have not been enough accurate measurements of surface areas in rabbits to make sure of the constant that should be used in the Meeh formula. Also the rabbit is peculiar in having a wide expanse of ear and a large cecum filled with inert material. Furthermore, data on the influence of body length, age, sex or other factors are entirely unknown. Probably the best that can be done is to choose the constant 10.8 suggested by Rubner for a rabbit without ears. The question of the absolute accuracy of the figures must await further researches. They will however at least give us valuable comparative results.

Using the formula

$$\text{Surface area (sq. cm.)} = 10.8 \times (\text{wt. in kgm.})^{\frac{2}{3}}$$

the average heat production for 76 tests on 36 rabbits was 32.73 calories per square meter per hour. This figure is within the range of those for other laboratory animals as well as that for man, and it therefore gives some confidence that the constant 10.8 has not been unwisely selected by Rubner. It is also an illustration of the universality of his law of surface

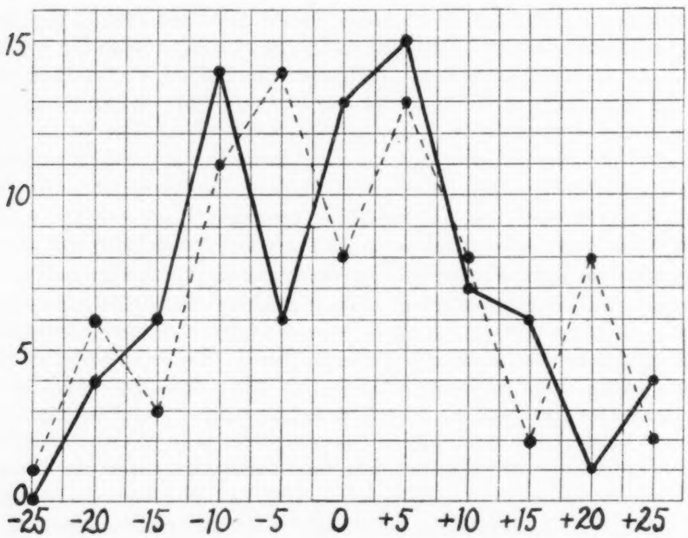

Chart 1. Abscissae—percentage variation from normal figure. 0 represents all tests within 2.5 per cent of the normal figure; 5, those within 2.5 per cent to 7.5 per cent of the normal; 10, those within 7.5 per cent to 12.5 per cent of the normal, etc.
Ordinates—number of cases.
Heavy line—curve for data calculated on a basis of surface area.
Broken line—curve for data calculated on basis of body weight.

area. In our series the average number of calories per square meter per 24 hours was 785. Over 75 per cent of all the determinations were within 12 per cent of this figure.

Chart 1 shows a graphic comparison of the two methods of calculation, i.e., surface area and weight, with regard to percentage variation from the normal figure. It is evident that within the weight groups used the two methods compare favorably.

Effect of pregnancy. The metabolism of 25 rabbits during 41 pregnancies

was determined. Observations were made throughout pregnancy and from two to three weeks post partum. These data are presented in table 1. It may be seen that during the first half of pregnancy there was little increase in metabolism, the rate from the 5th to the 15th day averaging only 2.63 calories per kilo per hour as compared with a normal of 2.61. The rate per square meter for the same period rose slightly from 32.73 to 33.32. Following this there was a noticeable progressive rise in the metabolic rate which reached a maximum of 3.19 calories per kilo and 41.5 calories per square meter just before parturition. This is an increase of 19 per cent in calories per kilo and 26 per cent in calories per square meter.

TABLE 1

(*a*) *Metabolism of 36 normal rabbits*

NUMBER OF TESTS	AVERAGE CALORIES PER KILOGRAM	AVERAGE CALORIES PER SQUARE METER
76	2.61	32.73

(*b*) *Metabolism tests on 25 rabbits during 41 pregnancies and the post partum period*

DAYS PREGNANT	NUMBER OF TESTS	AVERAGE CALORIES PER KILOGRAM PER HOUR	AVERAGE CALORIES PER SQUARE METER PER HOUR
1–10	31	2.59	32.99
5–15	30	2.63	33.32
11–20	23	2.73	36.71
16–25	40	2.91	37.83
21–30	59	3.15	40.89
26–30	38	3.19	41.50
DAYS POST PARTUM			
1–5	30	3.31	37.24
1–10	38	3.12	36.67
5–15	17	2.93	34.83
11–20	11	2.78	35.26
16–25	10	2.66	33.61

Following parturition there was first found to be a sharp drop and then a gradual return to the normal metabolic level which was reached after about twenty days.

In the above series, nine rabbits during twelve pregnancies were especially studied so that the total metabolism of the mother before parturition could be compared with the total of mother and young early in the post partum period. To do this it was necessary to determine the heat production of the young almost immediately after birth. The entire litter was wrapped in cotton and placed in a respiration chamber somewhat smaller than the one usually used. The cotton prevented any great loss of heat, and there was therefore little movement of the young animals.

The data from these experiments are presented in table 2. It may be seen first of all that there was no great deflection in the total energy production at birth. The total calories produced by the mother and young soon after birth differed only slightly from the heat production of the mother on the day before parturition. In eight experiments in which the metabolism of the young was determined by exact observation the difference did not exceed 2.35 calories per hour (or 20 per cent) and in five cases

TABLE 2

Twelve pregnancies in nine rabbits showing the mother's metabolism near the end of pregnancy, the combined metabolism of mother and young immediately after parturition, and the metabolism of mother and young taken separately

RABBIT NUMBER	METABOLISM NEAR END OF PREGNANCY		DURING EARLY POST PARTUM PERIOD						
			Metabolism of mother plus metabolism of young			Metabolism of mother		Metabolism of young	
	Days pregnant	Calories per hour	Days post partum	Total calories per hour	Per cent variation from that at end of pregnancy	Calories per hour	Fall in metabolism	Calories per kilogram	Calories per hour
5	29	11.87	1	11.84	−0.2	8.93	2.94	6.84	2.91
6	29	9.38	2	10.64	+13.4	7.93	1.45	7.88	2.71
6	28	11.33	1	13.68	+20.7	9.54	1.79	8.45	4.14
14	26	9.56	1	9.84	+2.9	6.74	2.82	10.50	3.10
14	28	8.38	1	8.59	+2.5	7.03	1.35	9.05	1.56
17	28	9.07	1	8.43	−7.0	7.52	1.55	8.25	0.91
26	29	9.49	1	8.99	−5.2	6.65	2.84	5.61	2.34
15A	29	8.67	1	10.39	+19.8	7.88	0.79	8.69	2.51
Four cases in which the metabolic rate of the young was not obtained. In these cases the average for the above 8 readings on the young was used, namely, 8.16 calories per kilo per hour.									
5	27	7.96	1	8.38	+5.2	5.77	2.19	8.16	2.61
15	29	8.58	1	9.01	+5.0	7.08	1.50	8.16	1.93
16	29	7.12	1	6.97	−2.1	5.91	1.21	8.16	1.06
23	29	8.55	1	9.37	+9.5	7.39	1.16	8.16	1.98

of the eight, the variation was only about a half a calorie or even less, or under 7 per cent. The results are in agreement with those of Carpenter and Murlin (1911) on women.

In four experiments it was impossible to get the heat production of the young. In these cases the weight of the litter has been multiplied by 8.16, the average rate per hour per kilo found in the other eight litters. That this figure is approximately correct is evidenced by the fact that the heat production thus determined when added to that of the mother during the

post partum period very closely approached the total heat production during the last day of pregnancy.

Since there is a loss of much inert material at birth and yet the total metabolism of mother and young immediately after birth remains the same as that of the pre-partum mother, there must be an increased rate of heat production somewhere in the mother-young combination. Although it is impossible to decide the point for the fetuses, there is a real increase for the post-partum mother. Having lost the fetal tissues as well as the accessory membranes and amniotic fluid she should be expected to produce the same number of calories per kilo or per square meter as in the normal state before pregnancy. As a matter of fact she is still producing some

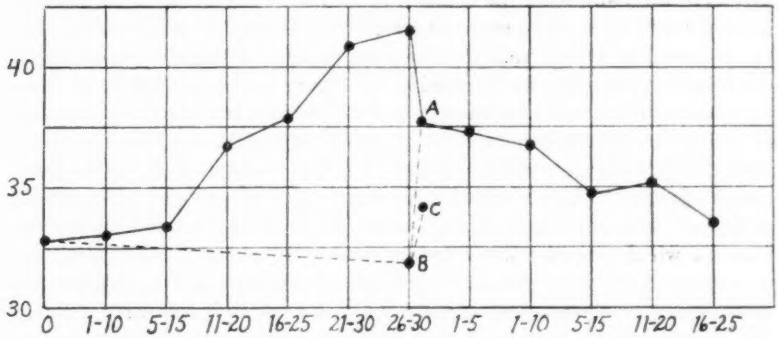

Chart 2. Abscissae represent days of pregnancy and the post-partum period. Ordinates represent calories per square meter per hour.

A, B and C represent the tests on the nine rabbits during 12 pregnancies which were especially studied.

A—Average rate of mother first day post partum.

B—Average rate of mother the day before parturition when combined surface area of mother and fetus was used.

C—Average rate of mother and young taken together the first day post-partum.

20 per cent more heat than before and she does not return to normal for about 15 days. There are at least three possible explanations for this higher post-partum rate, the activity of the mammary glands, involution products from the uterus or some unknown chemical product furnished by the fetal tissues and still remaining in the circulation. There is some indirect evidence that two of these factors may be operating. Our data show a return to normal in about 15 days. Involution of the uterus in the rabbit lasts about this length of time although it may be somewhat shorter in lactating rabbits. Rabbits lactate a month or even longer but after the young begin eating the drain on the mother is correspondingly lessened. These events occurring simultaneously may be supposed to have some causal relation to the return of the metabolic rate to normal.

Since Sandiford and Wheeler (1924) have given evidence in one human case during pregnancy that there is no increase in heat production per unit of active protoplasmic tissue, we have attempted to apply their method to our data with the hope of gaining information on this point. In order to do this, the following procedure was followed. First the weight of the new-born young was substracted from that of the pregnant mother just before parturition, and the resulting figure used in determining the surface area of the mother. The surface area of the newborn young was then calculated and added to that of the pre-partum mother, giving a figure which represented the combined surface area of mother and fetuses. There is no special formula known for estimating the surface area of fetal rabbits. We have therefore used the coefficient 10.8 which is the same as Rubner gives for rabbits without ears but which is considerably smaller than that of 12.5 used by Plaut (1921). The total number of calories produced by the pregnant organism was divided by the figure representing the combined surface area of mother and fetuses, and the result taken as calories per unit of active protoplasmic mass of the pregnant organism just before parturition. This procedure when applied to the aforementioned twelve pregnancies in nine rabbits resulted in the figure 31.8 calories per square meter of surface area which is not far from the figure of 32.7 for normal rabbits. Chart 2 which includes these figures shows that if the fetal surface area is considered, the heat production per unit of mother's active protoplasmic tissue is not increased in late pregnancy. Immediately following delivery the metabolism of the mother rises to 37.59 calories per square meter per hour, or about 15 per cent above the normal. Our data lead us to believe that the early post-partum metabolism per unit of surface area is higher than that either in the normal or late pregnant state. This increase as already mentioned is probably due to lactation, involution of the uterus or some unknown chemical influences remaining after parturition. As nearly as can be observed it does not seem to be due to increased activity of the mother.

CONCLUSIONS

1. During pregnancy in the rabbit there is a progressive increase in energy metabolism; on a basis of weight, from a normal of 2.61 calories per kilo per hour to 3.19 in the last 5 days of the gestation period; and on a basis of surface area, from 32.73 to 41.50 calories per square meter per hour.

2. The total metabolism is not markedly changed at birth; that is, the total heat production of the mother just before parturition is about equal to that of the mother and young combined just after parturition.

3. If the fetus be considered as a separate organism and its surface area plus that of the mother be used in computing the metabolism, no increase in rate is evident during pregnancy.

4. During the early post-partum period the metabolism of the rabbit, both per unit of weight and per unit of surface area is considerably above normal.

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THE INFLUENCE OF NUTRITION ON THE RESPONSE TO CERTAIN AMINO ACIDS

II. The Effect of Fasting Followed by Diets High in Carbohydrates

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In the first paper of this series, it was shown that the response to the intravenous administration of certain amino acids was constant in well nourished animals which were receiving a standard diet in sufficient amounts to maintain them at a constant weight, but that the nature of the response underwent certain definite changes during fasting. To recapitulate briefly, the changes that occurred during fasting were: 1, an increase in the specific dynamic action (the extra heat produced in excess of the existing basal value); 2, a decrease in the total heat production for the four-hour period after the administration of the amino acid; 3, a low respiratory quotient for the four-hour period after injection which does not rise above the fasting basal quotient; the average quotient after administration of the amino acids, in fifteen experiments performed on three animals during fasting was 0.693, and the corresponding basal quotients likewise averaged 0.691; this is in striking contrast to the results obtained in eleven experiments on the same animals while they were receiving the standard diet; under these conditions the respiratory quotients for the four-hour period after injection of the amino acid were usually slightly above the corresponding basal quotients; the latter averaged 0.77 and the former 0.82; 4, evidences of a toxic reaction characterized by nausea and retching in two of the animals but not in a third. This effect is practically never seen in normally nourished animals when an equivalent quantity of amino acid is administered, and 5, a suggestion that during fasting the animals do not deaminize as much of the administered amino acids, in the four-hour period after injection, as do the same animals when normally nourished. When the animals were returned to the standard diet after the fasting period it was found that there was a gradual return to the normal type of response which was approximately within normal limits after about nine days on the standard diet.

TABLE 1

Dog 3. Second fasting period followed by a diet high in carbohydrates, 3.69 grams glycine

		BASAL PERIOD (FOR EACH HOUR)				AFTER INJECTION FOR 4 ± 0.05 HOURS							
Conditions	Date (1928)	Total oxygen, liters	Protein nitrogen, gm.	Nonprotein respiratory quotient	Total calories	Total oxygen, liters	Protein nitrogen, gm.	Extra urea nitrogen glycine deaminized	Nonprotein respiratory quotient	Total calories	Total specific dynamic action	Specific dynamic action for each millimole glycine deaminized	Glycine unaccounted for during time of experiment, per cent
On standard diet	October 17	2.469	0.030	0.75	11.7	11.410	0.021	0.468 (68)*	0.80	54.8	7.8	0.23	25
On standard diet	October 19	2.562	0.027	0.74	12.1	11.780	0.119	0.536 (78)	0.78	56.0	7.4	0.19	17
Sixth day of fasting	October 25	2.241	0.062	0.72	10.4	11.337	0.096	0.464 (67)	0.71	53.0	10.7	0.32	25
Tenth day of fasting	October 29	2.106	0.099	0.66	9.7	12.615	0.414	0.617 (89)	0.70	58.6	19.0	0.43	4
Twelfth day of fasting	October 31	1.820	0.076	0.70	8.4	10.505	0.465	0.213 (31)	0.71	48.7	14.7	0.98	65
Fourteenth day of fasting†	November 2	1.890	0.122	0.66	8.8	10.686	‡	‡	0.75†	50.6	15.0	‡	
Third day on carbohydrate diet	November 5	2.503	0.023	1.01	12.5	10.957	0.119	0.045 (6.5)	1.00	54.9	4.3	1.4	89
Fourth day on carbohydrate diet	November 6	2.590	0.063	1.10	12.9	11.262	0.371	0.119 (17)	1.10	55.4	3.4	0.43	79
Ninth day on standard diet and syrup	November 16	2.571	0.094	0.80	12.2	11.556	0.589	0.123 (18)	0.91	55.4	5.4	0.60	76

* Numbers in parentheses indicate per cent of glycine which was deaminized during time of respiratory experiment.

† Seven grams glucose injected intravenously two hours before the glycine.

‡ Specimen lost.

Certain considerations, which need not be mentioned in detail, have suggested that these changes which were found to occur during fasting and subsequent realimentation with the standard diet were dependent on the amount of available carbohydrate present within the organism and that the active factor in the diet which was responsible for the changes occurring on refeeding, was the actual or potential carbohydrate content of the diet. In paper I and in table 1 of this paper two experiments are shown which were performed on two different animals on the thirteenth and fourteenth days of fasting, in which glucose was injected, in one experiment two hours before the administration of the amino acid and in another immediately after its administration. In these experiments there was a slight elevation of the respiratory quotient above the fasting level (0.74 and 0.75), but the total specific dynamic action did not decrease below the average value obtained during total fasting nor were the toxic symptoms prevented. These results showed that the presence of small amounts of circulating carbohydrate is not sufficient to alter the response and it was decided to carry out a series of experiments in which animals that previously had been fasted were fed carbohydrates almost exclusively for a few days in order to bring about deposition of carbohydrate in the various reservoirs of the body.

Method of experiments. The general procedures employed in administering the amino acids, in collecting the specimens of urine, in determining the respiratory metabolism and in making the secondary calculations from the data were the same as those outlined in the first paper, and the three animals used were the same.

Two satisfactory experiments were first performed while the animals were on the standard diet, the experiments being started about twenty-one hours after the last feeding. The animals were then fasted for periods varying from six to fourteen days, and during this period one or more experiments were performed. Immediately after the period of absolute fasting a diet high in carbohydrates was given, consisting of cracker meal and Karo syrup in various proportions, mixed with sufficient water to make a mush. This food was usually taken with avidity during the first few days, but in the later periods considerable coaxing and sometimes forced feeding was required to administer the prescribed amount. This diet was continued from four to eleven days, and during this period the response to the same quantity of the same amino acid was determined at frequent intervals.

Results of experiments. The changes in the response to the intravenous administration of alanine and glycine which resulted from a diet high in carbohydrates at the termination of a period of fasting, when compared with the response obtained in the same animal while on the standard diet or during fasting may be briefly summarized: 1, the total specific

dynamic action was reduced below the value obtained during fasting and in some instances was lower than the value obtained when the animal was on the standard diet; 2, the nonprotein respiratory quotient for the four-hour period after administration of the amino acid was definitely higher than the quotient after injection of the same amino acid while the animal was on the standard diet or during fasting; 3, the total heat production for the four-hour period after administration of the amino acid was somewhat irregularly influenced and in some instances was the same, above or below, the value obtained in the same animal when normally nourished; 4, the amount of amino acid apparently deaminized during the four-hour period after administration was also irregularly influenced by carbohydrate feeding, and 5, the toxic symptoms which frequently occur when amino acids are injected into fasting animals were prevented when sufficient carbohydrate was fed.

In the first paper, it was shown that during a thirteen-day fasting period there was no increase in the total specific dynamic action of the amino acid, phenylalanine, in contradistinction to the definite increase which was found to occur with alanine and glycine. The question naturally arose as to whether this difference in behavior was due to differences in the metabolism of phenylalanine or to some individual peculiarity of the particular animal used. In order to decide this point the same dog (dog 3) was injected with glycine during a fourteen-day fasting period (table 1). It was seen that the usual increase in the specific dynamic action occurred with glycine, the increases amounting to 41, 150, 93 and 97 per cent on the sixth, tenth, twelfth and fourteenth days of fasting. Also the nonprotein respiratory quotients for the four-hour periods after injection varied from 0.70 to 0.71, as was found in the other fasting experiments. Another point which seems worthy of mention is that when phenylalanine was used in this dog there was no evidence of a toxic reaction (nausea and retching) in any one of three experiments performed during thirteen days of fasting, but that a toxic reaction occurred in from thirteen minutes to one hour and seven minutes after injection in every experiment when glycine was used. From these results it appears likely that the difference in the reaction of the fasting animal to phenylalanine is the result of differences in the metabolism of the amino acid and not to any peculiarity of the animal.

When previously fasted animals were placed on diets high in carbohydrates the total specific dynamic action was always less than that obtained during total fasting, and was either reduced below the normal value obtained when the animal was on the standard diet (table 1 and fig. 1) or remained at approximately the normal level (fig. 2) in spite of the fact that the animals were on diets inadequate in all primary foodstuffs except carbohydrates. In the first paper it was shown that when the animals were returned to the standard diet after a period of fasting the total specific

dynamic action returned to approximately the normal value within nine days. In another experiment (table 1) it was found that if the standard diet was augmented by an extra feeding of Karo syrup in milk the total specific dynamic action was still below the normal value after nine days. This result may or may not be due to the extra carbohydrate fed, but

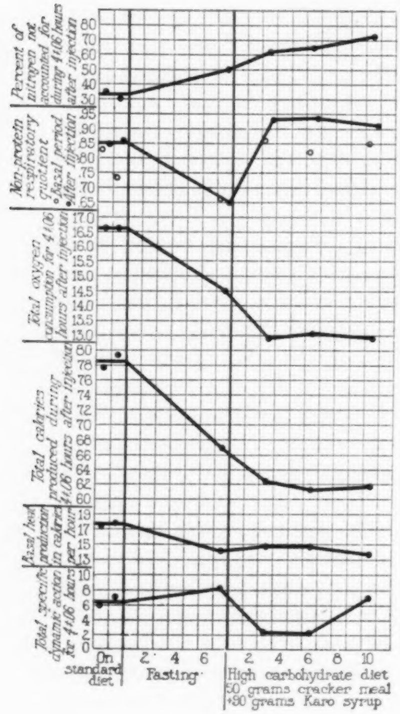

Fig. 1

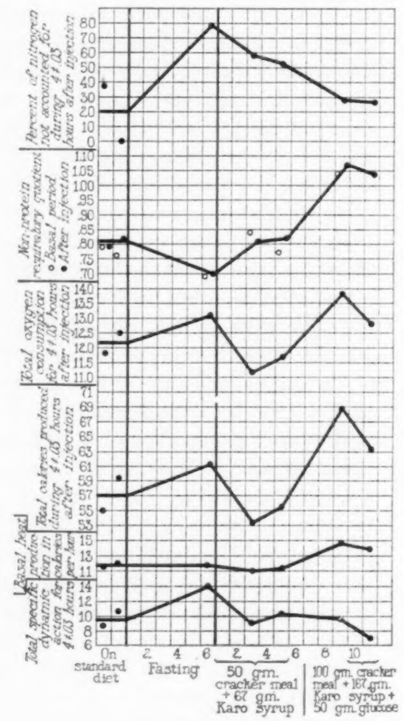

Fig. 2

Fig. 1. Second fasting period with dog 2. The response to 8.54 grams of alanine with the standard diet, on the seventh day of fasting and on the third, sixth and tenth days of a diet high in carbohydrates.

Fig. 2. Third fasting period with dog 1. The response to 5.08 grams of glycine with the standard diet, on the sixth day of fasting and on the third, fifth, ninth and eleventh days of a diet high in carbohydrates.

it is mentioned because it is in agreement with the other data on carbohydrate feeding. The injection of 7 grams of glucose two hours before the injection of the amino acid, on the fourteenth day of fasting, failed to reduce the specific dynamic action or to prevent the onset of toxic symptoms, but it did cause a slight rise in the respiratory quotient (table 1). This is

in harmony with a similar experiment reported in the first paper. The negative effect exerted by small doses of glucose given intravenously, when contrasted with the marked effect obtained when large amounts of carbohydrate were fed for several days, strongly suggested that the carbohydrate must become deposited in the cells in order to exert this specific effect.

The nonprotein respiratory quotient for the four-hour period after administration of the amino acids, in ten experiments performed on three previously fasting animals which were on diets high in carbohydrates averaged 0.95, and with two exceptions were 0.90 or above. This is in marked contrast to the average of 0.694 obtained in fifteen experiments on the same animals during fasting and 0.82 obtained in eleven experiments while they were on the standard diet. The value obtained for the respiratory quotients after injection of the amino acids appears to bear a direct relationship to the amount of carbohydrate fed on the days preceding the experiments and to some extent to the basal quotients; for example, with dog 3 (table 1) basal quotients of 1.01 and 1.10 were obtained on the third and fourth days of carbohydrate feeding and the corresponding quotients after injection of glycine were 1.00 and 1.10; the diet consisted of 150 grams of Karo syrup and 150 grams of cracker meal. This dog was then placed on the standard diet augmented with about 80 grams of Karo syrup, and on the ninth day of this diet the respiratory quotient after injection of glycine was 0.91 while the corresponding basal quotient was 0.80. With dog 1 (fig. 2) the quotients after injection of glycine on the third and fifth days of carbohydrate feeding were 0.81 and 0.83 and the corresponding basal quotients were 0.84 and 0.77 respectively; the diets preceding these two experiments were the same, namely, 50 grams of cracker meal and 67 grams of Karo syrup; on the ninth and eleventh days the quotients after administration of glycine were 0.107 and 0.104, the basal quotients being 1.04 in both instances; the carbohydrate content of the diets had been more than doubled and they were given late in the afternoon preceding the experiment. With dog 2 (fig. 1) the diet was constant and consisted of 50 grams of cracker meal and 90 grams of Karo syrup throughout the entire period. The quotients after injection of alanine likewise were constant, being 0.93, 0.93 and 0.91 on the third, sixth and tenth days respectively, with corresponding basal quotients of 0.86, 0.82 and 0.85. From these experiments it is clear that, in animals receiving diets high in carbohydrates, when the basal respiratory quotients are 1.00 or above, there is little if any change after the injection of alanine or glycine, but with basal quotients between 0.77 and 0.86 there occurs a definite rise after the administration of either of these amino acids.

The total heat production for the four-hour period after administration of the amino acid showed considerable variation as the result of realimentation with diets high in carbohydrates. With dog 2 (fig. 1) the total heat

production during the four hours after injection was lower on the carbohydrate diet than on the seventh day of fasting, but was constant from the third to the tenth day of carbohydrate feeding; the diet consisted of 50 grams of cracker meal and 90 grams of Karo syrup throughout the entire period. With dog 3 (table 1) the total heat production after injection on the third and fourth days of carbohydrate feeding was approximately the same as the normal value before fasting; the diet for this dog consisted of 150 grams of cracker meal and 150 grams of Karo syrup. With dog 1 (fig. 2) the total heat production after injection on the third and fifth days of carbohydrate feeding was only slightly below the normal value before fasting and the diet on these days consisted of 50 grams of cracker meal and 67 grams of Karo syrup. On the ninth and eleventh days the total heat production after injection was greatly increased and considerably exceeded the normal value before fasting; the diet corresponding to these two days was more than double that on the third and fifth days. From these relationships, it appears that the total heat production for the four-hour period after injection of alanine or glycine is roughly proportional to the amount of carbohydrate fed. In this connection it should be pointed out that dog 1 (fig. 2) on the sixth day of fasting, and dog 3 (table 1) on the tenth day of fasting showed higher values for the total heat production after injection than was found in the same animals while on the standard diet. These results are contrary to the results obtained in the other fasting experiments, in which the values showed a decrease during fasting; no explanation can be given for them.

The percentage of amino acid which was deaminized during the four-hour period after injection was irregularly influenced by carbohydrate feeding after a period of fasting. With dog 1 (fig. 2) the amount deaminized on the sixth day of fasting was definitely decreased below the normal but when the high carbohydrate diet was started there was a progressive increase in the amount deaminized, which reached the normal prefasting value in eleven days. With dog 3 (table 1) and dog 2 (fig. 1) the percentage of amino acid deaminized during the period of carbohydrate feeding was even less than during fasting. Since in one experiment a diet high in carbohydrates caused an increase in the amount deaminized and in two others a decrease, it was obviously impossible to draw conclusions relative to the influence of carbohydrate diets on deaminization under the conditions prevailing in these experiments.

One of the most interesting and striking effects of a carbohydrate diet on an otherwise fasting animal is the definite prevention of toxic reactions. For example, dog 3 (table 1) showed evidences of a toxic reaction, characterized by nausea and retching, in every experiment performed during fasting, and the time of onset of these symptoms varied in different experiments from thirteen minutes to one hour and seven minutes after injection. The

intravenous injection of 7 grams of glucose two hours before the injection of amino acid, on the fourteenth day of fasting, did not prevent or even delay the onset of symptoms, but they were totally abolished after the dog was given food high in carbohydrates for two days.

COMMENT. A diet high in carbohydrates for otherwise fasting animals brings about three definite changes in the nature of the response to the administration of the amino acids, alanine and glycine: 1, the total specific dynamic reaction is definitely lowered below the value obtained during total fasting, and in some instances may be lower than the value obtained while the animal is on the standard diet; 2, the respiratory quotients for the four-hour period after injection are considerably higher than corresponding quotients obtained during fasting or on the standard diet and are always elevated above the respective basal quotients except when the latter are 1.00 or above, and 3, the toxic symptoms which frequently follow the injection of alanine or glycine in fasting animals are abolished.

The facts as presented are definite but their interpretation is difficult. Several hypotheses could be advanced to explain the observed changes, but since none is supported by sufficient experimental proof, it is unnecessary to comment on them here.

The results of these experiments also have brought out one other significant point: the respiratory quotient after administration of an amino acid bears no relation to the theoretic respiratory quotient of the amino acid given, but is entirely dependent on the nutritional condition of the animal. Thus, during complete fasting, glycine with a theoretic quotient of 1.00 and alanine with a theoretic quotient of 0.83 both produce an increase in metabolism with quotients approximating 0.70, whereas if carbohydrate is fed to otherwise fasting animals the resulting quotients may range from 0.81 to 1.10, depending on the amount of carbohydrate given.

Honda determined the specific dynamic action of meat in rats on three different diets: bacon fat; bread, milk and glucose, and finally meat and peptone combined with injections of phlorhizin. He found that on the fat diet the specific dynamic action was very low, on the meat and peptone diet combined with phlorhizin it was very high, and the values obtained with the animals receiving the carbohydrate diet were intermediate. It is rather difficult to compare his experiments with those reported by us since total fasting is not strictly comparable with the feeding of bacon fat, and a fasting period followed by a diet high in carbohydrates is not comparable to the condition produced when an otherwise well nourished animal is given extra carbohydrate in the diet. His results also suggest that perhaps not enough carbohydrate was fed greatly to alter the nature of the response.

SUMMARY

The administration of a high, almost exclusive carbohydrate diet at the end of a fasting period causes a marked change in the nature of the response to the intravenous administration of the amino acids, alanine and glycine. These changes consist in: 1, a reduction of the specific dynamic action below the fasting value and at times even below the value obtained in the same animal when receiving a normal diet; 2, a high respiratory quotient for the four-hour period after injection of the amino acid which is dependent on the amount of carbohydrate fed; in ten experiments on three otherwise fasting animals on carbohydrate diets the average respiratory quotient was 0.95, with maximal and minimal values of 0.81 and 1.10; in all but two experiments the quotients were 0.90 or above; when the basal quotients were 1.00 or above, there was no further elevation after administering the amino acids, but when the basal quotients were between 0.77 and 0.86 there was always a definite rise in the quotients for the four-hour period after injection; 3, prevention of the toxic reactions which often follow the intravenous administration of alanine or glycine to the fasting animal, by carbohydrate feeding but not by the injection of small quantities (7 or 12.5 gm.) of glucose either before or just after the injection of the amino acid, and 4, somewhat irregular influence on the total heat production and the amount of amino acid deaminized during the four-hour period following administration of the amino acids; this possibly depends on the fact that under these conditions the animal is on a somewhat unstable nutritional balance at some intermediate point between the well nourished and the totally fasting condition.

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THE EFFECT OF ANOXEMIA ON HUNGER CONTRACTIONS

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It is a well known physiologic fact that the stomach may show definite hunger contractions when it is empty. Owing to the importance of commercial aviation it was thought well worth while to study the effect of low oxygen tension on hunger contractions. While there is already considerable literature on physiological changes in the body brought about by diminished oxygen tension, as far as we know no work has been reported on alimentary tract motility.

Methods. Dogs were trained to lie quietly in the same position for three or four hours and were then taught to swallow a rubber tube with a small balloon attached. Young and vigorous dogs were chosen for these experiments. They were not fed for 24 to 48 hours before the experiment. The method which was used to study hunger contractions is that described by Carlson (1916). A balloon was inserted into the animal's stomach and by means of a small stomach tube was connected to a water manometer, which in turn was allowed to write on a smoked drum. Approximately 5 to 10 cm. of water pressure were used.

A mask was put over the dog's muzzle, such as is described by Kunde (1923). One alteration, however, was made. A small hole was made in the side of the muzzle and a brass pipe was soldered into this hole so that it projected about one-half inch inside the muzzle: the stomach tube was attached to this and to the part of the pipe which projected on the outside of the muzzle the water manometer was attached. This was necessary for the reason that the animal was to breathe from a closed system.

In order to produce a low oxygen tension a nitrous-oxide oxygen apparatus was used as described in a previous paper (Van Liere, 1927). The oxygen was diluted with nitrogen and the apparatus could be so adjusted that the percentage of oxygen could be varied at will. This apparatus was attached to the muzzle so that the dog breathed from a large rubber bag. A flutter valve was placed between the latter and the muzzle so that the expired air would not enter the otherwise closed system.

During the control periods the animal was allowed to breathe a mixture of gases, the oxygen percentage of which was approximately 20 per cent.

The apparatus could be adjusted easily so as to vary the amount of oxygen without disturbing in any way the rest of the apparatus.

Results. When the animal was allowed to breathe a mixture of nitro-

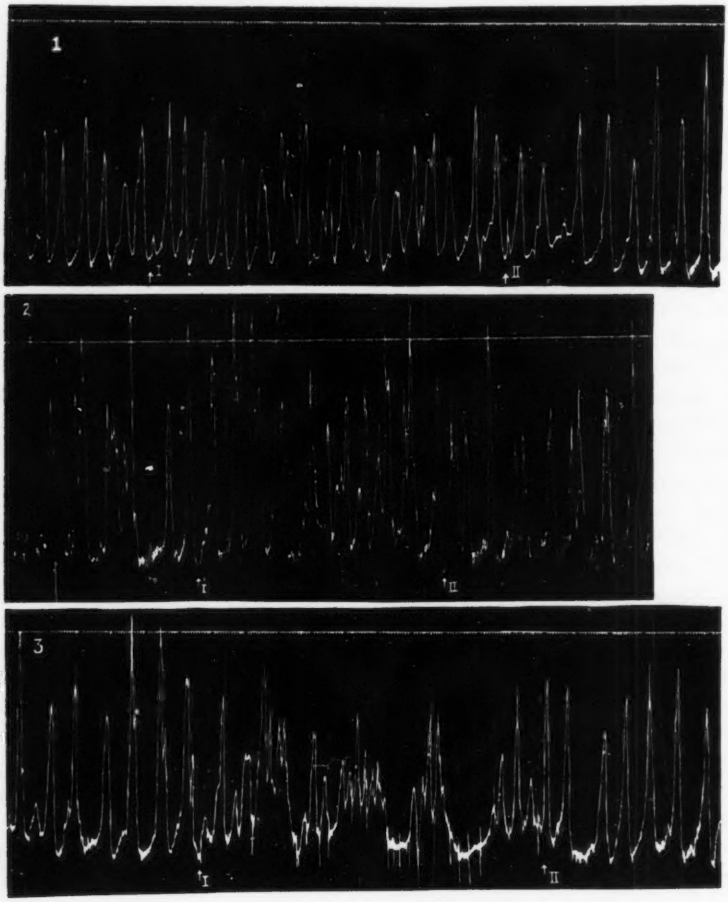


Fig. 1. The effect of 10 per cent oxygen tension on hunger contractions. At *I* the oxygen tension was reduced from 20 per cent to 10 per cent. At *II* a 20 per cent oxygen tension was restored. Time interval, 5 seconds.

Fig. 2. The effect of 8 per cent oxygen tension. At *I* the oxygen tension was changed from 20 per cent to 8 per cent; at *II* from 8 per cent to 20 per cent.

Fig. 3. The effect of 7 per cent oxygen tension. At *I* the oxygen tension was changed from 20 per cent to 7 per cent; at *II* from 7 per cent to 20 per cent.

gen and oxygen, the latter being in a concentration of 10 per cent, a slight but distinct rise in gastric tone occurred. Lower percentages of oxygen produced a greater rise in tone. If the percentage of oxygen breathed was 8 per cent or less, the hunger contractions definitely decreased in height. The hunger contractions persisted even when the oxygen tension was 5 per cent.

After the animal had been subjected to a short period of anoxemia and was again allowed to breathe a mixture of gases which contained 20 per cent of oxygen or atmospheric air, hunger contractions became very feeble and the gastric tone decreased. This may be called a post-anoxemic effect. The contractions would then gradually increase in height so as to produce what appeared like a staircase effect and would often become higher than the control.

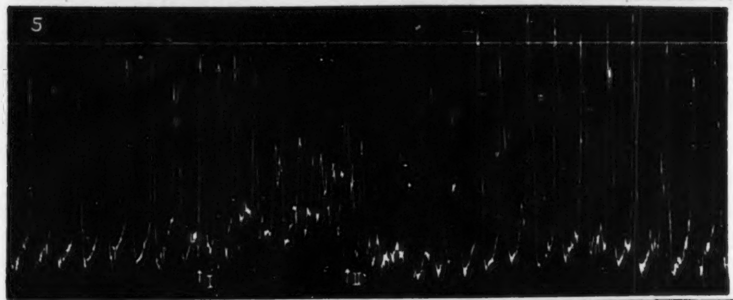
Discussion. It would seem from the above results that gastric tone is first affected when the oxygen tension is approximately 10 per cent. This may be called then the threshold—it corresponds to about one-half atmosphere. This varies somewhat, however, in different animals and we have reason to believe that it also depends upon the general condition of the animal. On one or two occasions the animals were suffering from a diarrhea which distinctly lessened their ability to withstand anoxemia.

If the animals were allowed to breathe a mixture of the gases in which the oxygen tension was less than 10 per cent, the rise in gastric tone became more marked and the height of the hunger contractions was distinctly diminished. If the oxygen tension was lower than 5 per cent the animals became restless within two or three minutes and it was impossible to keep them quiet; therefore, lower percentages of oxygen were not used as it would have been difficult to interpret the results owing to skeletal movements.

It is interesting to note that the hunger contractions were not abolished even when the animals were permitted to breathe a mixture of gases containing only 5 per cent of oxygen. This would correspond to an altitude well over 30,000 feet, which is about the upper limit of endurance in unacclimatized animals. They would lie quietly for only three or four minutes at this degree of anoxemia and the respirations of course were markedly accentuated, but seemingly these exaggerated respiratory movements would cause no skeletal-visceral reflex which inhibited the hunger contractions.

It has been shown by Koehler et al. (1925) that in animals subjected to anoxemia the pH of the blood rises and that there is a definite alkalosis. This is doubtless due to the hyperventilation. This period, however, lasts for only about 15 minutes and it is followed by a marked acidosis, depending upon the degree of anoxemia. In the experiments reported in this paper the animals were subjected to anoxemia for periods from 10 to

15 minutes. They would, therefore, fall within this period of alkalosis. It was shown furthermore by McSwiney and Newton (1927a) on isolated



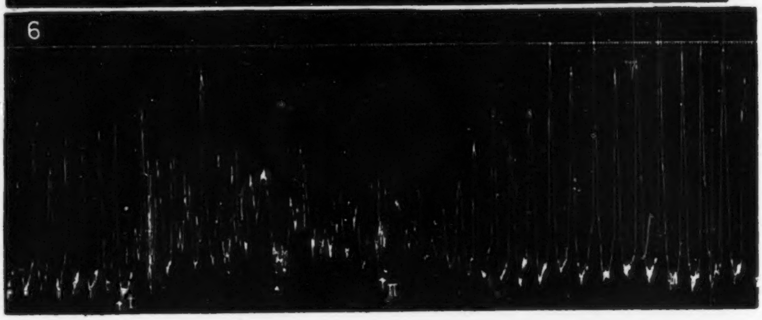


Fig. 4. The effect of 6 per cent oxygen tension. At *I* the oxygen tension was changed from 20 per cent to 6 per cent; at *II* from 6 per cent to 20 per cent.

Figs. 5 and 6. The effect of 5 per cent oxygen tension. At *I* the oxygen tension was changed from 20 per cent to 5 per cent; at *II* from 5 per cent to 20 per cent.

Note particularly the post anoxemic depression followed by a staircase-like effect.

strips of the intestines that alkalosis caused a rise in tone and the same authors in a subsequent paper (1927b) showed that there was a diminution

in the height of contractions in isolated smooth muscle strips. This may explain the results obtained in our work.

Greene and Gilbert (1922) have shown that degrees of anoxemia comparable to those used in this experiment tend to produce vagospasm. The results reported here may simply be a reflection of this effect on the tone and motility of the empty stomach.

What may be termed the post-anoxemic effect is an interesting phenomenon. There is a distinct post-anoxemic depression during which time the hunger contractions may practically be abolished and the gastric tone may come back to normal or even fall below normal. This post-anoxemic effect may, of course, be influenced or caused by a lag in the asphyxial effects, because we term anything post-anoxemic after we have established the normal oxygen tension in the inspired air. The hunger contractions after this period gradually increase in height, producing a staircase-like effect.

This paper apparently contains no definite evidence concerning the loss of hunger in mountain sickness. It is said (Air Service Medical, 1919) that unacclimatized people are often sick at an altitude of 10,000 feet, and that most people are sick at 14,000 feet and that practically everyone is sick at 18,000 feet. The last figure corresponds to about 10.50 per cent oxygen. The oxygen per cent on top of Pike's Peak is about 12. In dogs under resting conditions no perceptible change was discerned in the hunger contractions at 12 per cent oxygen. Carlson, however, states that in certain pathological conditions normal hunger contractions may cause a feeling of faintness and nausea and it is possible that in mountain sickness we are dealing with such a condition. It would seem thus that the loss of hunger in mountain sickness is produced by a change in the central nervous rather than in the peripheral hunger mechanism.

SUMMARY

1. Gastric tone is increased at an oxygen tension of 10 per cent or less. The rise in gastric tone varies directly with the degree of anoxemia.

2. At 8 per cent oxygen tension or less the hunger contractions are distinctly diminished in height. They persist to as low an oxygen tension as the unacclimatized animal can withstand which is usually from 4.5 to 5 per cent.

3. There is an apparent inhibition of hunger contractions and gastric tone immediately following the period of anoxemia. After this period of depression the hunger contractions become gradually higher and as a rule exceed the control.

4. This problem suggests that changes in the central nervous mechanism are responsible for the loss of hunger in mountain sickness.

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THE MECHANISM OF EPINEPHRINE ACTION

VI. Changes in Blood Sugar, Lactic Acid and Blood Pressure During Continuous Intravenous Injection of Epinephrine[1]

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The influence of epinephrine injections on blood lactic acid has been investigated in this laboratory on two previous occasions. In the first paper (1) 7 experiments (3 on rabbits and 4 on cats) were recorded in which the subcutaneous administration of epinephrine produced an increase in blood lactic acid. It was also noted that a hyperglycemia produced by other means (i.e., glucose feeding) did not cause significant changes in the lactic acid content of the blood. In the second paper (2) the relation between the sugar and lactic acid curves in blood was studied in detail following the subcutaneous injection of 0.2 mgm. of epinephrine per kilo, and lactic acid was also determined in arterial and venous blood of the leg. In the latter case the rabbits were under amytal narcosis and therefore in a state of complete muscular relaxation, but the increase in blood lactic acid was of the same magnitude as in unanesthetized animals. Since venous blood of the leg contained more lactic acid than arterial blood, it was concluded that muscle glycogen was the source of the increase in blood lactic acid and it was pointed out that previous observations of a decrease in muscle glycogen (3) were in best harmony with this assumption.

In the present experiments epinephrine was administered by means of a continuous intravenous injection. This has several advantages over the subcutaneous form of administration. First, the rate of entrance of epinephrine into the blood stream is not only known but can be kept constant. Secondly, the minimal rate which is still capable of producing changes in either blood sugar or lactic acid can be determined, and thirdly, since the administration of epinephrine can be terminated at will, observations as to the rate at which blood sugar and lactic acid return to normal can be made. Finally, rates of injection which lead to changes in blood sugar and lactic acid can be compared with rates which produce changes in blood pressure.

[1] Papers I to V appeared in The Journal of Biological Chemistry, 1928–1930.

EXPERIMENTAL. *Changes in blood sugar and lactic acid.* Healthy, well-nourished rabbits of 2.2 to 3.1 kilos in weight were used. After a fasting period of 24 hours the animals were placed in a wooden box just large enough to allow them a comfortable position and a preliminary blood sample of 2 cc. was removed from the marginal ear vein. Shortly afterwards the desired dilution was prepared from the 1 to 1000 adrenalin stock solution of Parke, Davis & Co. and the injection was started. A needle connected by a good length of rubber tubing with the burette containing the epinephrine solution was introduced into the marginal ear vein and held in place by two soft bulldog clamps. This made it unnecessary to fasten the ear or to interfere in any way with the movements of the animal. As a rule the rabbits remained very quiet throughout the experimental period. An even flow of the solution from the burette was maintained by means of the device of Burn and Dale (4). Generally from 13 to 15 cc. were injected per hour. During the period of infusion which lasted two hours blood samples were removed every half-hour and one or two blood samples were taken after the infusion. Blood sugar and lactic acid were determined in duplicate in the same tungstic acid filtrate, the former by means of the Hagedorn and Jensen (5) and the latter by means of the Friedemann, Cotonio and Shaffer method (6) as modified by Friedemann and Kendall (7). In a few cases blood taken during epinephrine hyperglycemia was analyzed after fermentation with yeast but no marked changes in the non-sugar reducing substances were noted. The values ranged between 30 and 38 mgm. per cent and the blood sugar values recorded in figure 1 and table 1 are therefore to this extent too high.

The dilutions of epinephrine varied between 1 to 75,000 and 1 to 2,000,000 and the rates of infusion between 0.001 and 0.00005 mgm. of epinephrine per kilo per minute.

Attention is called to the control experiments which serve as base line for the curves in figures 1 and 2 and which show that the procedure used (infusion of 30 cc. salt solution in 2 hours, repeated blood sampling, etc.) causes no significant changes in either blood sugar or lactic acid. An inspection of figures 1 and 2 shows which rates of infusion of epinephrine produce an appreciable rise in blood sugar and lactic acid. In order to find the minimal rate of infusion which has this effect, the figures in table 1 should be examined. There can be little doubt that a rate of injection of 0.0001 mgm. per kilo per minute produces significant changes in blood sugar and lactic acid. In both of the experiments recorded in table 1 the average increase during the infusion is much greater than the changes observed in control experiments performed under the same conditions. The rate of infusion of 0.00005 mgm. per kilo per minute seems to be effective as far as blood sugar is concerned, since the average increase of 9 and 19 mgm. respectively, when contrasted with a fall in blood sugar in

one control experiment and an average increase of 3 mgm. in the other control experiment, is quite definite. The same may be said about the increase in blood lactic acid in one experiment. Nevertheless, since the changes in blood sugar and lactic acid are slight, it may be debated whether this rate of injection should be regarded as definitely effective.

The foregoing analysis shows that the minimal rate of infusion which is still capable of influencing the carbohydrate metabolism of the unnarcotized rabbit is between 0.0001 and 0.00005 mgm. of epinephrine per kilo per minute and it is of importance that not only the blood sugar but also

TABLE 1

Blood sugar and lactic acid (in milligrams per 100 cc.) before and during intravenous injection of epinephrine

	BEFORE INJECTION	DURING INJECTION		RATE OF INJECTION
		Average value	Highest value	
Blood sugar	127* 125**	215 183	220 197	0.0001 mgm. per kilo per minute
Lactic acid	11.1* 10.2**	15.9 21.5	24.4 25.9	
Blood sugar	141 124	150 143	156 149	0.00005 mgm. per kilo per minute
Lactic acid	11.6 5.5	15.2 9.9	16.9 12.4	
Blood sugar	122 113	119 116	122 120	Control experiments
Lactic acid	5.9 6.6	4.5 5.6	5.5 6.3	

* Experiment A of figures 1 and 2

** Experiment B of figures 1 and 2.

(The other experiments are grouped in the same manner.)

the blood lactic acid level is influenced by such a rate of injection. The blood lactic acid level reacts as sensitively to epinephrine as the blood sugar level and an increase in the former is therefore as characteristic of epinephrine action as an increase in the latter.

According to Trendelenburg (8) rates of infusion of 0.000028 to 0.000047 mgm. of epinephrine per kilo per minute are without influence on the blood sugar level of unanesthetized rabbits, while rates of infusion of 0.00006 to 0.0001 mgm. per kilo per minute produce hyperglycemia without glycosuria. These figures are fully confirmed by the present investigation. Determinations of blood lactic acid were not made by this author.

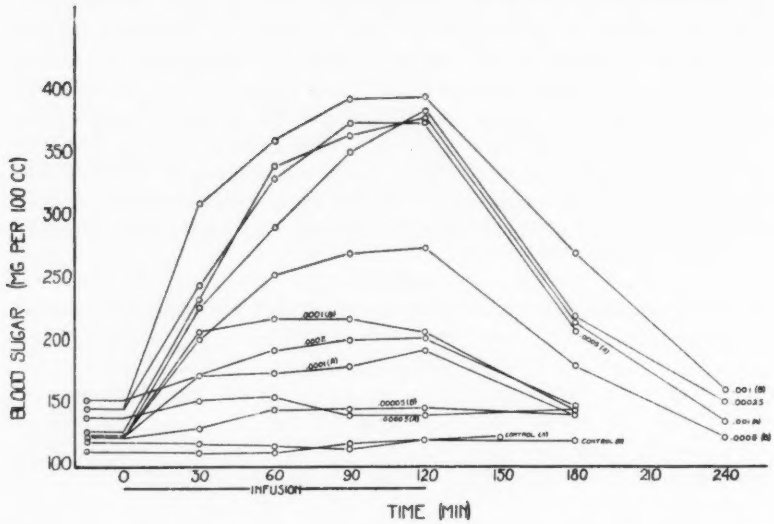

Fig. 1. Changes in blood sugar of normal rabbits during continuous intravenous injection of epinephrine at rates ranging from 0.00005 to 0.001 mgm. per kilo per minute. The corresponding values for blood lactic acid are shown in figure 2.

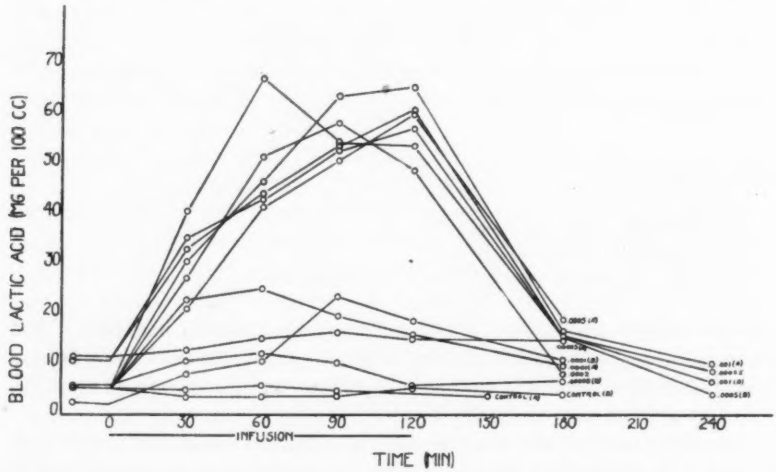

Fig. 2. Changes in blood lactic acid of normal rabbits during continuous intravenous injection of epinephrine at rates ranging from 0.00005 to 0.001 mgm. per kilo per minute. The corresponding values for blood sugar are shown in figure 1.

The shape of the curves in figures 1 and 2 is determined by the rate of production of blood sugar and lactic acid in relation to the rate of removal of these substances from the blood. The rate of production depends among other factors upon the percentages of liver and muscle glycogen and it is known that both show marked variations in rabbits fasted previously for 24 hours. Since an immediate effect of epinephrine is a diminution in the utilization of blood sugar in muscle (2), the rate of removal of blood sugar will be largely determined by the degree of this inhibition and if there is glycosuria, by the rate of excretion of sugar in the urine. Removal of lactic acid depends to a considerable extent upon the activity of the liver, since it has been found that a large part of the lactic acid formed

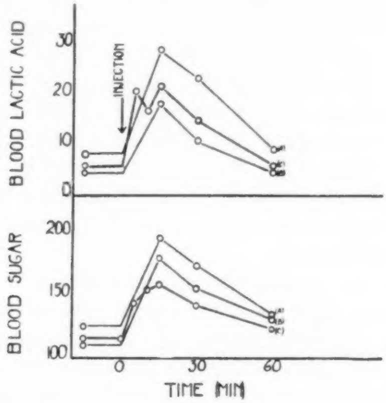

Fig. 3. Effect of a sudden intravenous injection of 0.03 mgm. of epinephrine per kilo on blood sugar and lactic acid of normal rabbits.

during epinephrine action is deposited as liver glycogen (3). With so many factors entering into production and removal of blood sugar and lactic acid, individual differences in the shape of the curves and in the response to the same epinephrine dose are to be expected. It seems in general that with low rates of infusion (0.00005 to 0.0002 mgm. per kilo per minute) the curves for both blood sugar and lactic acid flatten out as the administration of epinephrine proceeds. Assuming that the rate of production remains the same, this would mean that the rate of removal increases in later periods of infusion. With high rates of injection (0.0002 to 0.001 mgm. per kilo per minute) the production of blood sugar and lactic acid greatly exceeds their removal and the curves for both continue to rise, in some cases in the form of a straight line, while in others there is some flattening out after 30, 60 or 90 minutes.

A point illustrated in the curves in figures 1 and 2 is that blood sugar and

lactic acid increase only as long as epinephrine enters the blood stream. As soon as the infusion is stopped, both blood sugar and lactic acid begin to fall, at rates which are often surprisingly uniform in the different experiments. This lack of after-effect of the epinephrine injection is due to the rapid destruction of the hormone in blood and tissues and for the same reason a sudden intravenous injection even of a large dose has relatively little effect on blood sugar and lactic acid. In figure 3 which is drawn on the same scale as figures 1 and 2, three experiments are reported in which 0.03 mgm. of epinephrine per kilo was injected at once into the blood stream. This is the same amount of epinephrine which is administered in the course of 1 hour at a rate of infusion of 0.0005 mgm. per kilo per minute. Two examples of the effects produced by this rate of infusion are shown in figures 1 and 2. Nothing could be more striking than the difference in the response to the same amount of epinephrine when the injection is made at different rates. In the case of the sudden injection of 0.03 mgm. per kilo, blood sugar and lactic acid do not rise higher than 190 and 29 mgm. per cent respectively; they begin to fall 15 minutes after the injection and return to normal in 1 hour. On the other hand, when 0.03 mgm. of epinephrine is injected in the course of 1 hour, blood sugar and lactic acid continue to rise while the injection is being made and reach values of 380 and 64 mgm. per cent respectively.

The long drawn out hyperglycemia and increase in blood lactic acid after subcutaneous injection speak for a prolonged and therefore for a slow rate of absorption of epinephrine from the subcutaneous tissue. In a previous paper (2) the average glycemic and lactic acid curves obtained in rabbits following the subcutaneous injection of 0.2 mgm. of epinephrine per kilo were reproduced. When these curves are compared with those shown in the present paper, it becomes evident that they are not of the type shown in figure 3 but resemble those obtained in the experiments in figures 1 and 2 with rates of infusion of 0.0005 to 0.001 mgm. per kilo per minute. The resemblance to the effects produced by a continuous intravenous infusion is not a coincidence but is due to the following facts. Since after the subcutaneous injection of 0.2 mgm. of epinephrine per kilo the hyperglycemia persists for 4 hours and since epinephrine produces hyperglycemia only as long as it enters the blood stream, the absorption of epinephrine from its subcutaneous depot must have persisted for at least 4 hours. The maximal rate of absorption, assuming complete absorption in 4 hours, would therefore be $\frac{0.2}{240} = 0.0008$ mgm. per kilo per minute. A somewhat lower figure is arrived at when the blood pressure response is used as indicator of the rate of absorption from the subcutaneous tissue, which makes it very probable that absorption is not completed in 4 hours. In rabbits under amytal anesthesia one generally observes a

rise in blood pressure when epinephrine is injected intravenously at a rate of 0.0005 mgm. per kilo per minute (table 2). Since after the subcutaneous injection of 0.2 mgm. per kilo a rise in blood pressure is not observed under any conditions, the rate of absorption must be below 0.0005 mgm. per kilo per minute. The lack of blood pressure response after subcutaneous injection could also be due to rapid destruction of epinephrine in the subcutaneous tissue, but in this case it would be difficult to explain why the

TABLE 2

Determination of the smallest rate of injection of epinephrine causing a rise in the blood pressure of rabbits

EXPERIMENT NUMBER	INITIAL BLOOD PRESSURE	RATE OF INJECTION	HIGHEST BLOOD PRESSURE	INCREASE	REMARKS
	mm. Hg	*mgm./kilo/min.*	*mm. Hg*	*mm. Hg*	
1	119	0.00045	120	+1	Unanesthetized
	120	0.0009	131	+11	Unanesthetized
	110	9.0018	140	+30	Unanesthetized
2	120	0.00045	127	+7	Unanesthetized
	118	0.0009	136	+18	Unanesthetized
3	110	0.00045	112	+2	Unanesthetized
	112	0.0009	119	+7	Unanesthetized
4	101	0.0004	100	−1	Unanesthetized
	99	0.0008	112	+13	Unanesthetized
	102	0.0012	120	+18	Unanesthetized
5	108	0.00025	112	+4	Amytal
	106	0.0005	128	+22	Amytal
6	110	0.0004	118	+8	Amytal
	105	0.0008	121	+16	Amytal
7	119	0.0005	124	+5	Amytal
	122	0.001	133	+11	Amytal
8	96	0.00045	104	+8	Amytal
	96	0.0009	114	+18	Amytal

hyperglycemia persists for 4 hours and it would also be impossible to account for the fact that Luckhardt and Koppanyi (9) obtained a rise in blood pressure many hours after the injection when they massaged the injected area. These points have been discussed at some length because they do not seem to be generally accepted. For a further discussion reference is made to a previous article (10).

Changes in blood pressure. The main object of the blood pressure ex-

periments was the desire to know what relation existed between the minimal rate of infusion of epinephrine affecting blood pressure and the minimal rate of infusion causing an increase in blood lactic acid. For this

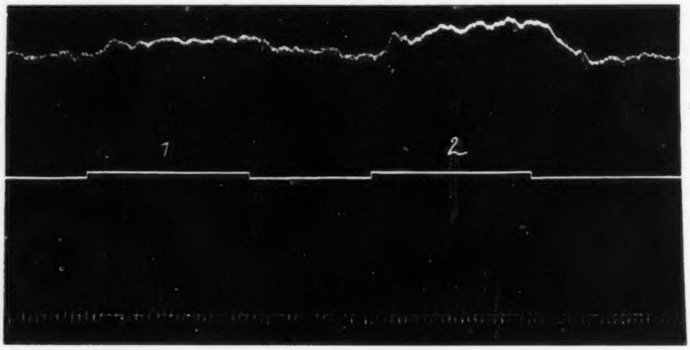

Fig. 4. Experiment 2 in table 2. Unanesthetized rabbit. Time in 5 seconds at base line of mercury manometer.

1, 1 cc. of 1 to 500,000 in 2 minutes or 0.00045 mgm. of epinephrine per kilo per minute.

2, 1 cc. of 1 to 250,000 in 2 minutes or 0.0009 mgm. of epinephrine per kilo per minute.

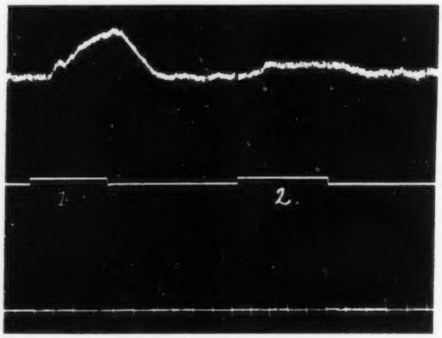

Fig. 5. Experiment 5 in table 2. Male rabbit under amytal anesthesia. Time in 30 seconds at base line of mercury manometer.

1, 1 cc. of 1 to 500,000 in 2 minutes or 0.0005 mgm. of epinephrine per kilo per minute.

2, 1 cc. of 1 to 1,000,000 in 2 minutes or 0.00025 mgm. of epinephrine per kilo per minute.

Subcutaneous injection of 0.2 mgm. of epinephrine per kilo was without effect on blood pressure.

purpose it was necessary to work on the unanesthetized animal. Blood pressure tracings from the carotid artery of the unnarcotized rabbit were made in the following manner. In a preliminary operation under light ether anesthesia the carotid artery was exposed, a loop of catgut was placed around it and the skin was closed with clips. Four to five hours later the clips were removed, the carotid artery was pulled to the surface by means of the catgut loop and a cannula of the usual type was introduced. The cannula was held in place by means of strips of adhesive tape. While this was being done the animal was sitting on the lap of an attendant who bent the head of the animal backward. It was always easy to introduce the cannula, because the procedure did not inflict any pain. The animals were placed in a small wooden box in which they remained in a normal sitting position as long as the experiment lasted. The box was provided with a sliding door which made it easy to reach the cannula and to establish connections with the mercury manometer. Three per cent sodium citrate was used as the anticoagulant and the cannula itself was filled with a strong solution of heparin. Injections of epinephrine, lasting for 2 minutes and timed by the beats of a metronome, were made into the marginal ear vein. Some blood pressure records were obtained while the animals were under amytal, anesthesia being produced by means of intravenous injection of the drug. The anesthesia was light in all cases. In each of the 8 experiments performed (4 in unanesthetized animals and 4 under amytal) the minimal rate of injection of epinephrine causing a rise in blood pressure was determined. This is shown in table 2 and two typical examples are reproduced in the curves in figures 4 and 5. According to Trendelenburg and Fleischhauer (11) a rate of infusion of 0.0007 to 0.0008 mgm. of epinephrine per kilo per minute is just able to raise the blood pressure of unnarcotized rabbits. In the experiments in table 2 the rates of injection leading to a rise in blood pressure were greater (0.0008 to 0.0009 mgm. per kilo per minute), possibly because the initial blood pressure was 20 to 35 mm. Hg higher than in the experiments of the above mentioned authors. A fall in blood pressure, attributable to the injected epinephrine, was not observed on any occasion, whether the animals were unanesthetized or under the influence of amytal. A given rate of injection either produced no change or a rise in blood pressure. It will be noted in table 2 that the blood pressure of the animals under amytal anesthesia reacts to intravenously injected epinephrine with a greater sensitivity than that of unanesthetized animals. An analysis of this observation is however beyond the scope of this paper. The main result is the following. Since definite changes are produced in blood sugar and lactic acid by rates of injection of 0.0001 mgm. per kilo per minute, the minimal rate of injection influencing carbohydrate metabolism is at least 8 times smaller than the minimal rate affecting the blood pressure

of unnarcotized rabbits. It is understood that such rates of injection, even if they do not influence blood pressure, may nevertheless change the amounts of blood passing through different regions of the body by causing vasoconstriction in one part and vasodilatation in another part. Since small doses of epinephrine are supposed to cause a vasodilatation in muscle, the suggestion made by Macleod (12) that the increase in blood lactic acid is due to asphyxia in muscle, has little likelihood. Campos, Cannon *et al.* (13) suggest that the increase in blood lactic acid after epinephrine injections which they confirm, may be due merely to a washing out of lactic acid from muscle on account of the improved circulation. This is rendered improbable by the observation that muscle glycogen diminishes (3) and that a lactic acid concentration higher in venous than in arterial blood of the leg persists for hours after the subcutaneous injection of epinephrine (2), an effect which cannot be explained by washing out but requires an increased rate of production of lactic acid. Cannon and Rapport (14) found an average maximal discharge of epinephrine from the adrenals of 0.003 mgm. per kilo per minute which is 30 times larger than the rate of injection which raises blood sugar and lactic acid. In view of this, increased lactic acid formation in resting muscle may be regarded as one of the physiological effects of epinephrine injection and it may be anticipated that the same is true for epinephrine discharged from the adrenals.[2] Experiments are now in progress to study the finer mechanism of this lactic acid formation.

SUMMARY

1. Observations have been made as to the changes produced in blood sugar and lactic acid of normal rabbits during continuous intravenous injection of epinephrine at rates ranging from 0.00005 to 0.001 mgm. per kilo per minute. The minimal effective rate of injection of 0.00005 to 0.0001 mgm. per kilo per minute produces an increase not only in blood sugar but also in blood lactic acid. This rate of injection is 30 times smaller than the maximal rate of discharge of epinephrine from the adrenals as recorded by Cannon and Rapport and 8 times smaller than the rate of intravenous injection which causes a rise in blood pressure of unanesthetized rabbits. In view of these facts the increase in blood lactic acid after epinephrine injections is regarded as physiologically significant.

2. As soon as the injection is discontinued, both blood sugar and lactic acid begin to fall, showing that epinephrine is destroyed rapidly and that the after effect is of short duration. These points are also illustrated in experiments in which 0.03 mgm. of epinephrine per kilo was injected at

[2] It has since been found that piqûre produces a rise in blood lactic acid coincident with the rise in blood sugar.

once into the blood stream. After such an injection the peak value of blood sugar and lactic acid is reached in 15 minutes or sooner and there is a return to normal in 1 hour. This is in contrast to the sustained and to the much larger increase in blood sugar and lactic acid to be observed when the same amount of epinephrine is injected in the course of 1 hour.

3. The similarity between the sugar and lactic acid curves in blood observed after subcutaneous and after continuous intravenous injection of epinephrine is emphasized.

4. The smallest rate of injection of epinephrine which causes a perceptible rise in the blood pressure of unnarcotized rabbits is close to 0.0008 mgm. per kilo per minute.

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THE POTENTIAL ANALYSIS OF THE TURTLE AND CAT SYMPATHETIC AND VAGUS NERVE TRUNKS

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Action potentials from autonomic nerves here studied, recorded at some distance from the point stimulated, show three or four main potential elevations indicative of corresponding groups of nerve axons whose impulses have different rates of propagation. The first elevation alone, when present, appears to correspond in rate to the group of waves of Gasser and Erlanger (1922, 1924).[2] A second and slower component is prominent in most autonomic nerves and presumably corresponds to the delta of sciatic nerves. A third still slower component is sometimes fused with this into a B elevation. Since these components are readily separable by other criteria than conduction rate they are designated as B_1 and B_2 respectively. A fourth component is always present as a C elevation and has a conduction rate approximately that reported by Chauchard (1925) for unmyelinated axons. The wave originally designated as Delta by Erlanger (1927), and since it travelled at a rate slower than the sizes of the myelinated axons present would lead one to expect, was assigned by him, tentatively, to unmyelinated axons. More recently (Erlanger and Gasser, 1930) this potential has been assigned to the main elevation termed B. Each of these potentials is further divisible into poorly separable waves, somewhat as the A elevation consists of the Alpha, Beta and Gamma waves in the sciatic.

These groups of waves vary in amplitude characteristically from nerve to nerve, and certain are absent from the record of certain nerves, in such a manner that the potential record becomes an index of the nerve's fiber content. Finally when one group of potentials is absent from the record, one type of axon is absent from the histological cross section, by which correlation the type of axon responsible for each of these groups of waves

[1] The facilities for carrying on this investigation were supplied by the Department of Physiology until these became available in the Department of Surgery.

[2] Gasser and Erlanger occasionally found in the frog sciatic a minute delta wave; their experience with higher amplification now inclines them to disregard this wave as a feature of the first or *A* elevation.

may be identified. The investigation was undertaken as a preliminary to a study of autonomic nerve functioning in the body, from the point of view of interpretation of visceral function. Certain other nerves have been compared with these as the progress of investigation seemed to warrant. Preliminary accounts of parts of this investigation have appeared previously (Heinbecker, 1929 and Heinbecker and Bishop, 1929).[3]

I. MATERIALS. The turtles used were Pseudemys elegans, Pseudemys concinnia, Pseudemys scripta and Chrysemys marginata. The results in all were similar. For practical purposes the Chrysemys marginata was found the most satisfactory because it has in a much larger percentage of cases a cervical sympathetic trunk entirely separate from the cervical portion of the vagus. The nerves employed were the cervical portion of the vagus and the portions of the cervical sympathetic trunk proximal and distal to the superior cervical ganglion. In the cat the sympathetic trunk proximal to the inferior cervical ganglion was used and that portion of the vagus which approximated it.

TECHNIQUE. The nerves were removed from the body after pithing the turtles, or in the case of the cats, under ether, and most of the connective tissue was dissected off; removal of the outer common sheath enclosing both nerves generally allowed their complete separation as separate trunks. Usually no signs of fiber injury were detectable after this dissection, and any nerves showing injury or relative depression of function were discarded. The nerves were kept in Ringer's solution at room temperature or preferably in the ice box until used, some of them as long as three hours. A slow depression of function was observed, chiefly as slowed conduction rates, but slightly, if any more, than takes place in frog nerves. This appears sufficient cause for differences of rate of corresponding processes in different nerves, but may not be the only one. Oxygen was usually supplied to both warm and cold blooded nerves. The turtle nerves were observed at room temperature (20–25 degrees), the cat nerves at 35 to 37 degrees. Nonpolarizable calomel electrodes were usually used for both stimulating and recording, but it has been found that recording electrodes may be of metal, when leading into the amplifiers employed, with no

[3] In these preliminary accounts, the first part of the B elevation has been referred to as a part of an A process. In the interest of consistency in nomenclature, we now recognize four components of the potential of a complex nerve record, which, at the distance of conduction usually available, are distributed to give two or three main elevations or groups of waves, *A*, *B*, and *C*. The second component previously referred to as *A* is thus included in the *B* elevation, as B_1. This change in nomenclature, of course, indicates no changes in the experimental findings previously reported. Further differentiation is, of course, possible, and the terminology employed is more or less arbitrary. Differentiation between these components of potential, referred to below, is more fully discussed in a forthcoming paper.

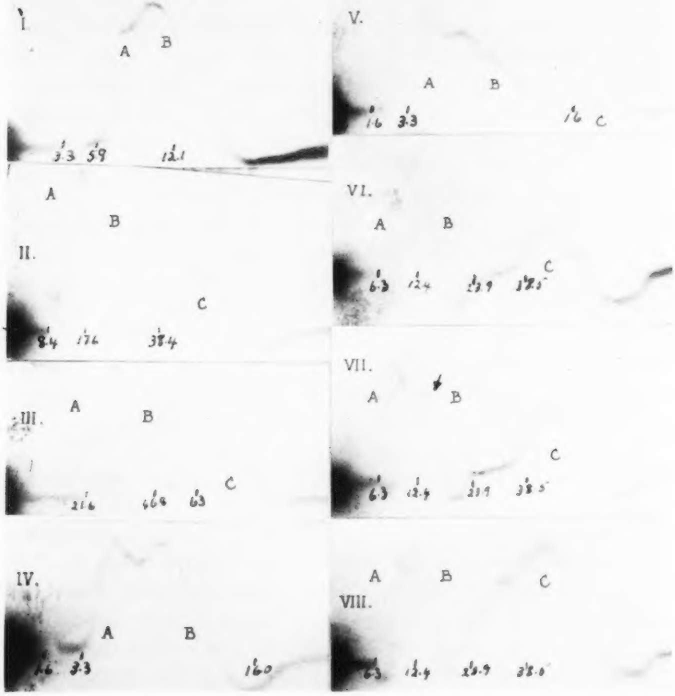

Fig. 1. Film I. Conducted action potential, turtle vagus, showing the first and second potentials marked B_1 and B_2; also start of third potential C. Time in sigma, conduction distance 28 mm., temperature 24 degrees centigrade.

Film II. Conducted action potential same nerve showing three potentials marked B_1, B_2 and C. Note moderate diphasity of record. Nerve killed half way between lead electrodes.

Film III. Conducted action potential, above nerve, after burning close to ground electrode. Note record is now apparently monophasic, but the potential is lower.

Film IV. Conducted action potential turtle sympathetic showing first and second group potentials marked B_1 and B_2. Time in sigma, conduction distance 18 millimeters, temperature 24 degrees Centigrade.

Film V. Conducted action potential same nerve as in IV with similar time distribution after burning close up to ground electrode. Note again the change from slight diphasity of film IV to monophasity.

Film VI. Conducted action potentials of same nerve partially diphasic as in Film VI showing the three group potentials marked B_1, B_2 and C; stimulus a short galvanic current comparable to a shock.

Film VII. Conducted action potentials same nerve with stimulus lasting to end of first group potential (notch between B_1 and B_2). Note a slight widening of the

appreciable distortion of the potential picture through polarization. It is much more essential that stimulating electrodes be non-polarizable, to avoid shock distortions.[4]

Stimulation was timed at a rate of one to five per second, or single stimuli were used, the makes from an induction coil being, of course, below threshold for threshold response of a given fiber to the break shock. Condenser charges were also employed, the discharges being rendered ineffective (Bishop and Heinbecker, 1930). In most cases the stimulating electrodes were connected into one arm of a Wheatstone bridge to obviate large escapes of current from the stimulating circuit into the leads (Bishop, 1927); galvanic currents of short duration were also employed as stimuli in some cases, using this circuit to test for repetitive responses as confusing elements in the record. Potentials were observed and recorded by means of the cathode ray oscillograph and amplifier arranged much as previously described (Gasser and Erlanger, 1922) and at a sensitivity of 150 to 200 millimeters per millivolt. Most of the cat nerve records were taken with an arrangement of apparatus (Bishop, 1929) in which an approximately linear time line was obtained by means of a condenser charged through a vacuum tube, the whole being controlled by a pendulum interruptor.

All histological material was fixed and stained in one per cent osmic acid for forty-eight hours It was then imbedded in paraffin or preferably double imbedded in celloidin and paraffin and cut three to five mu in thickness.

II. FORM OF THE CONNECTED ACTION POTENTIAL OF THE VAGUS AND CERVICAL SYMPATHETIC NERVES OF THE TURTLE. Typical conducted action

[4] In recording action potentials from nerve it is perhaps more important to employ nonpolarizable electrodes as stimulating leads than as leads to the recording apparatus. This is especially true when recording through leads into an apparatus which draws little current, such as a vacuum tube grid circuit, and when stimulating by the strong currents necessary to excite the less irritable fibers of a nerve. We have not been able to detect any difference between records made through platinum or other metal as compared with nonpolarizable electrodes, but a pair of platinum stimulating electrodes have a polarizable "capacity" several times as large as that of the average nerve which is being stimulated, and this capacity unless compensated in a bridge arrangement causes a large "escape" distortion, even at a distance along the nerve.

potential in the B group designated by an arrow. This is interpreted as the result of a repetitive process arising in the fibers giving rise to the first group potentials.

Film VIII. Conducted action potential similar to that of film VII but stimulus lasting to almost the middle of the third group potential. Note the increase in amplitude of the wave previous to and including the main C potential wave. This is interpreted as the result of repetitive processes arising in the first or second fiber groups.

All the records of this paper were obtained with the stimulating electrodes in a bridge-circuit to reduce the stimulus escape (Bishop, 1927).

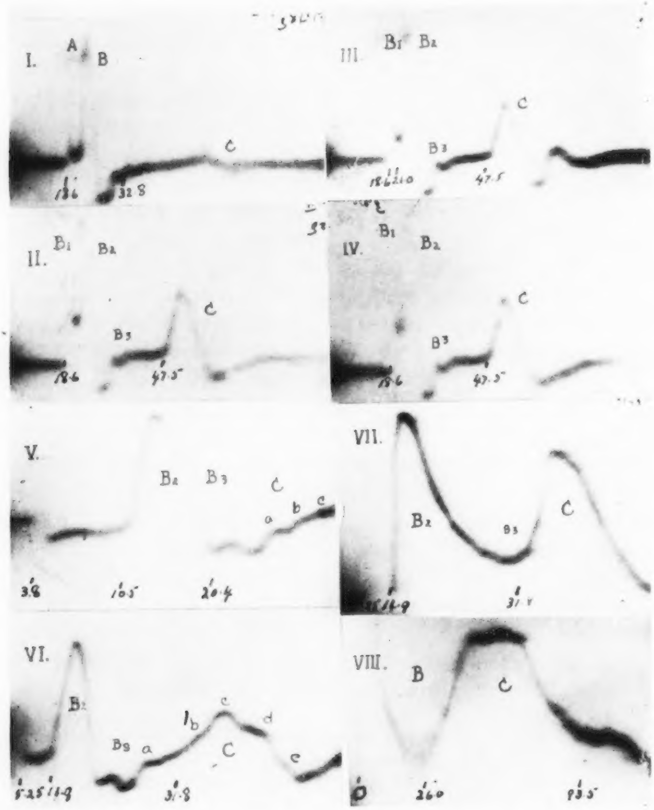

Fig. 2. Films I, II, III and IV. Conducted action potentials turtle sympathetic with increasing duration of galvanic stimulus. The first break in the line preceding the B_1 and B_2 elevations is the residual of escape. Note that in the first film only the first and second group processes are prominent. In the second film as a result of the longer period of stimulation the third group potential is also present. In film III with a still longer duration of stimulus a deflection later than the main C potential wave is seen. Film IV with still further increase in duration of stimulus is practically identical with film II. The small wave marked B_3 in this and subsequent records has a threshold nearer to the main B wave than to the C and is, therefore, considered part of the B_2 elevation. It also appears in the cat nerves. Time in sigma (all records). Conduction distance 15 mm. Temperature 22.8 degrees Centigrade.

Film V. Conducted action potential turtle sympathetic showing only the B_2 potential with the commencement of the C. No B_1 elevation is present in this nerve. Note the presence of three distinct maxima in the C potential group marked a, b and c. Conduction distance 20 mm., temperature 22 degrees Centigrade.

potentials of the vagus and cervical sympathetic nerves of the turtle are shown in the photographic records of figures 1, 2 and 3. In the vagus nerve two main elevations of potential are almost invariably present, consisting of the second, third and fourth potential components, the first, characteristic of the sciatic, being absent. In the turtle it is found that the second component also may occasionally be absent or very small, and then this component runs in a separate nerve comparable to the depressor of other animals. This condition is much more prevalent in turtle studied having only the last two components well developed. In other respects the conducted action potentials of the two nerves are comparable if one leaves out of consideration changes dependent upon differences in the relative number of fibers giving rise to each action potention group. These three potentials are designated as the B_1, B_2 and C elements, without, however, any prejudice as to homologies of function in different nerves with similar potential forms.

Each of these main potentials also exhibits secondary action potential maxima. These are usually four in number in the vagus for the B_1 division, each with definite thresholds and conduction rates. In the B_2 division there are usually three such secondary potential maxima and in the C also three, sometimes five. Within the various groups, the secondary maxima are presumably in the main expressions of the different conduction rates of fibers of differing size, as in the Alpha, Beta and Gamma waves of the frog sciatic (Erlanger and Gasser, 1924). These potentials are thus expressions of fiber groupings of slightly different size and numbers in a continuous size distribution curve. By changing the duration of a galvanic stimulus or altering its intensity the fiber group maxima can be readily demonstrated. In figure 2 is shown the separation of certain of these maxima by simply changing the duration of such a stimulus.

It is not to be inferred that each action potential wave is the result of only one type of fiber. Evidence has been obtained especially in the cat that the groups overlap. Certain fibers in the B_1 group travel more slowly than the fastest fibers of the B_2 group. Consequently the potential waves

Film VI. Conducted action potential of the same nerve with a slower deflection to show especially the third potential group. Note the presence of eight potential maxima marked *a*, *b*, *c*, *d*, *e*, *f* and *g*, the nerves being somewhat diphasic which emphasizes the distinctness of the waves.

Films VII and VIII. Conducted action potentials corresponding to those of films V and VI but after heating close up to the ground electrode. Note the fusion of the potential maxima so that now only the two main group potentials with no special subgroup maxima are shown. This illustrates the greater ease with which potential maxima can be demonstrated in partially diphasic records. In film VIII there are also seen potentials which are considered the result of repetitive processes. For further discussion see text. All potentials obtained by use of short galvanic stimuli.

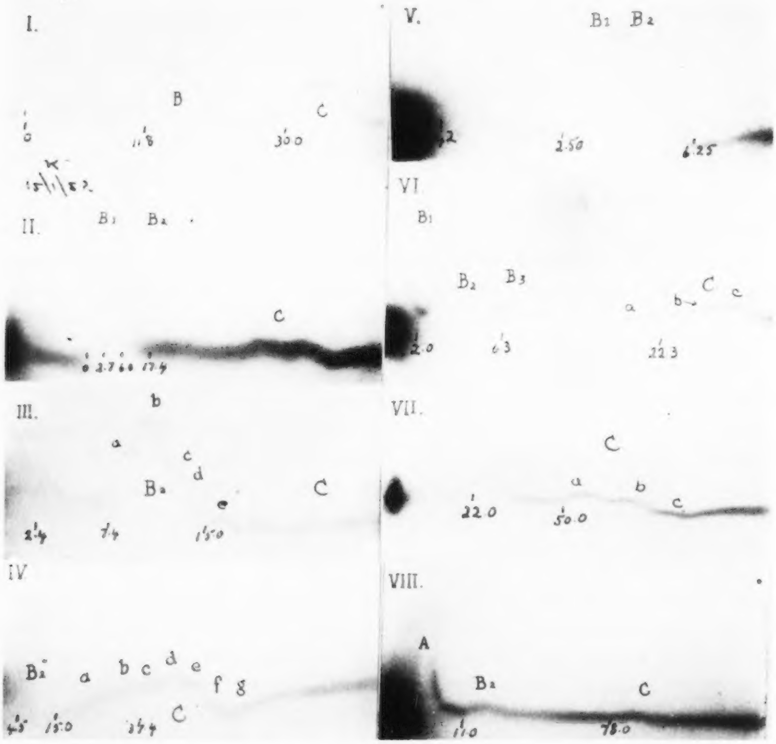

Fig. 3. Film I. Conducted action potential cervical sympathetic of the turtle showing only the two late potential groups. Conduction rate first process of group B_2 3.8 m. per second, first process group C 1.5 m. per second. The cross section of the nerve used in this experiment is shown in figure 8, A and C. Time in sigma (all records). Conduction distance 45 mm. Temperature 23 degrees Centigrade.

Film II. Conducted action potential cervical sympathetic showing the main group potentials marked B_1 B_2 and C. With a faster line B_1 and B_2 separate distinctly as in previous records. Conduction rates first process group B_1 8.3 m. per second, group B_2 3.8 m. per second, group C 0.57 m. per second. The cross section of the nerve giving rise to this action potential is shown in figure 8, B and D. Conduction distance 23 mm., temperature 23.5 degrees Centigrade.

Film III. Conducted action potential cervical sympathetic illustrating potential group B_2 and five potential maxima of this group marked *a*, *b*, *c*, *d*, and *e*. Conduction distance 17.5 mm.

Film IV. Conducted action potential same nerve as in film III with slower deflection to show the potential maxima of group C, marked *a*, *b*, *c*, *f*, and *g*.

Film V. Conducted action potential vagus nerve illustrating potential maxima of the first two groups, marked B_1 and B_2.

Film VI. Action potential of same nerve as in film VI with slower time distribu-

of the slower of the B_1 fibers would appear beneath the potential wave of the B_2 fibers, the same relationship applies to the other potentials. While there appears to be a marked interval between the maxima of the B and C groups still in certain nerves, especially the vagi of both turtle and cat, where satisfactory records are obtained, there is a continuous potential throughout the record, with, however, certain conspicuous maxima. The thresholds of these groups appear to be separated more sharply than their conduction rates.

III. Potential of the conducted impulses in the vagus and sympathetic nerves of the cat. The form of the potential record of the sympathetic myelinated fibers of the cat after conduction (fig. 4, records 5 and 8) is quite different from that of the sciatic. A bullfrog sciatic as conventionally recorded is included (fig. 4, record 4) for comparison. It was chosen because the Gamma and Delta waves were exceptionally well developed. In contrast to this, the first waves of the sympathetic are low and the total amplitude increases with time during the record, this situation corresponding to the disproportionately large number of small fibers which are known to be present in the sympathetic as compared with the sciatic. The first potentials of this nerve correspond approximately with the slowest A waves of the frog sciatic or the B wave of the dog's saphenous. Comparison of the sympathetic records, in which an A elevation is absent, with that of the sciatic, illustrates the fact that the upper limit of the B elevations overlaps the lower limits of the A, a circumstance illustrated further in the vagus records, where the potential does not return to zero between A and B. Between the start of this record and the last of the potentials recorded ascribed to myelinated axons from six to nine maxima in a continuous distribution may be distinguished, depending upon the distance of conduction, the separation into discrete waves being much less complete than in the sciatic. The potential processes appear more discrete, however, on the oscillograph while the record is being observed as the stimulus is altered, a gradual increase in strength of the latter causing periodic, and in places, rather abrupt increases in area. They are also easy to distinguish in diphasic records (figs. 2 and 6) .The records of figure 4 were taken at the strengths of stimuli just preceding

tion showing especially the first and second group potentials with considerable of group C potential.

Film VII. Conducted action potential same nerve as in film VI to show all of the C potential, the subgroups of which are marked, a, b, and c. The thresholds and conduction rates of the group potentials and their subdivisions for films V, VI and VII are given in table 1.

Film VIII. Conducted action potential turtle sciatic to illustrate three main groups potentials A, B_2 and C. Conduction rates of the main process group A 19 m. per second group B_2 3.04 m. per second, group C 0.43 m. per second. Conduction distance 33.5 mm. Temperature 28 degrees Centigrade.

these more abrupt increases. By subtraction of these records one from the other the approximate areas and forms of the group potentials can be obtained, but in any area resulting, there might be distinguishable more than one potential maximum. No significance can be assigned at present to these secondary maxima.

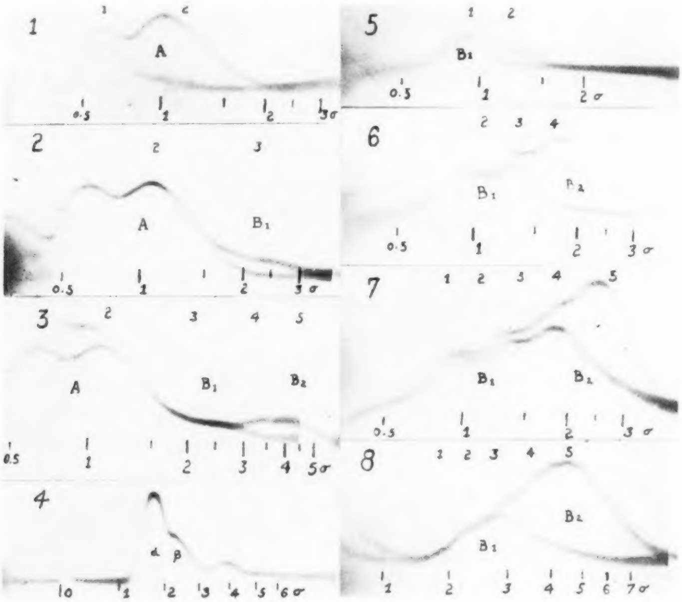

Fig. 4. Films I, II, III. Cat vagus, conduction distance 38 mm. Temperature 35.5 degrees Centigrade; rate of first process 80 M.P.S. of fifth, 11 M.P.S.

Film IV. Bullfrog sciatic, conduction distance 63 mm. Temperature 27 degrees Centigrade; rate of first process 51 M.P.S. of fourth 13 M.P.S.

Films V, VI and VII. Right cat sympathetic, conduction distance 17 mm. Temperature 37.5 degrees Centigrade; rate of first process 28 M.P.S.; of fifth 8.5 M.P.S.

Film VIII. Left sympathetic of same cat, conduction distance 30 mm. Temperature 36 degrees Centigrade; rate of first process 20 M.P.S.; of fifth 8.3 M.P.S. Nerve out of body six hours.

Repeated stimuli, 5 per second, amplification record 4, 6 mm/mv, for the rest, 50 mm/mv. Records superimposed in printing so that last of base line and first significant wave superimpose. As the stimulus becomes stronger to bring in later waves, the first part of the record is progressively distorted in spite of a larger part, the escape distortion being balanced out in a Wheatstonebridge at the stimulus. Times measured from shock which is off records to the left in most cases. The fifth were so numbered in each record is inferred to be the B_2 wave, although the B_2 process may contribute to the fourth.

In the vagus record (figs. 4 and 5) the main difference in potential form is due to the presence of two groups of large fibers whose electrical processes travel more rapidly than any in the sympathetic and which thus give rise to an *A* elevation. Each of these processes can generally be differentiated into at least two waves poorly separated out. Between these and the groups duplicating the sympathetic picture there is a relative but not an absolute interval.

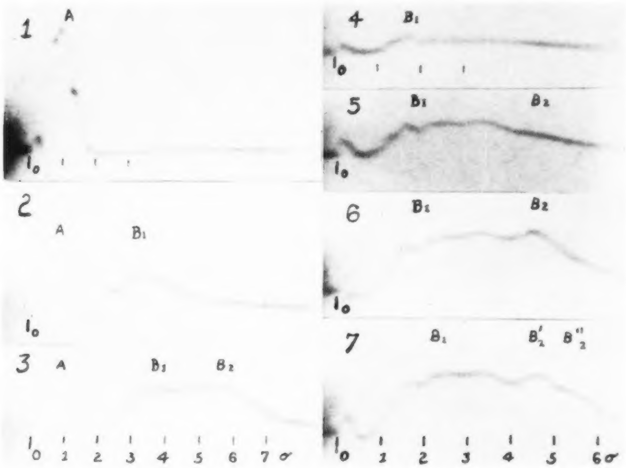

Fig. 5. Films I, II and III. Cat vagus, conduction distance 45 mm. Temperature 37.5 degrees Centigrade. Rate of first process 100 M.P.S., rate of second or B_1 process 25 M.P.S., rate of B_2 process 10 M.P.S.

Films IV, V, VI and VII. Cat sympathetic, conduction distance 31 mm. Temperature 36 degrees Centigrade. Rate of first process (B_1) 30 M.P.S., rate of B_2 8.5 M.P.S.

Repeated stimuli, linear time record, balanced circuit for stimulus. There is a relatively greater interval in strength of threshold between B_1 and B_2 elevations than there is in conduction rates.

Occasionally also in the sympathetic, a small wave appears ahead of of the main B_1 elevation, in approximately the position of the second wave of the vagus (fig. 6, record 1). In the vagus, on the other hand, the increase in amplitude of the first part of the *B* elevation does not occur so strikingly as in the sympathetic.

Comparing these potentials with those of the frog sciatic (fig. 3) the fiber groups may be homologized schematically as in the diagram of figure 7 (see section 3). Taken as a whole, *A* and *B* potential elevations of the vagus may be separated into a number of elements each complex, but more

sharply separated off from the next than are the components of any one element from one another. The first two potential maxima always present in the vagus and the second only occasionally in the sympathetic, may be homologous (i.e., in form or distribution of fibers) to the alpha and beta waves of the sciatic and thus constitute the A elevation of the vagus. The reason for such comparison is that the larger fibers which presumably

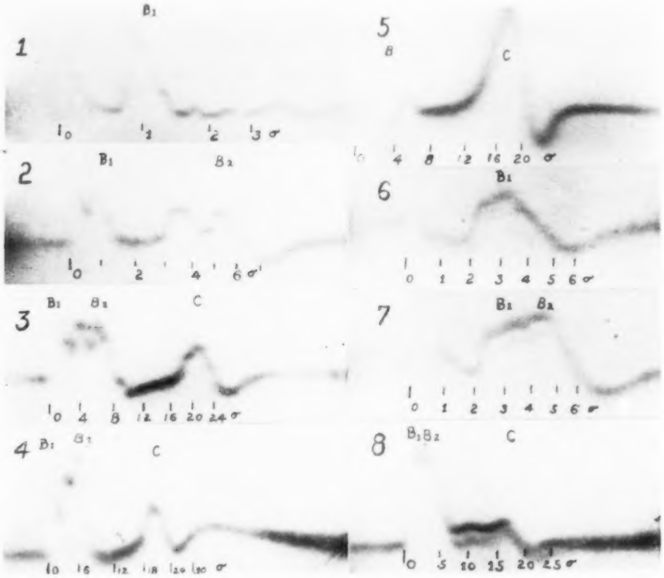

Fig. 6. Films I, II, III, IV. Cat sympathetic, conduction distance 30 mm. Temperature 37 degrees Centigrade. Rate of first (B_1) process 38 M.P.S., of B_2 6.6 M.P.S., of C main wave, 1.75 M.P.S. The C process lasts much longer than this main wave upon strong stimulation. The wave preceding that marked B_2, about 10 M.P.S., may also be part of the B_2 elevation, in which case the usual B_1 component of the sympathetic is anomalous in this nerve, the first spike of the B_1 being more rapid in conduction than usual, and the rest of the B_1 almost completely absent.

Film V. C wave of a vagus nerve from the same cat, the A and B_1 components with the shock, lying in the poorly exposed fast part of the record, and passing off the oscillograph upwards.

Films VI, VII, VIII. Cat sympathetic, B_1 and B_2 showing almost no interval between them, a small wave B_3 preceding the main C elevation. See also record 3, this figure, and figure II, for the small wave, which is usually present, though disguised in monophasic records.

This figure as compared to the two previous illustrates the advantages of a partially diphasic record for differentiating the potential elements in the complete picture.

give rise to the first wave in the vagus separate out, in part, at least, to the motor branches that supply sketetal muscle. No homology as to function in the body is suggested other than this.

The third and fourth maxima, in the vagus, usually present also as the first two to the sympathetic, constitute the first division of the *B* elevation, i.e., the second or B_1 potential component of the complete record. Each usually shows two waves. The next division of the potential, the B_2 component, follows the last rather closely, and is usually distinctly double in both nerves. While this B_2 element is presumably due to myelinated axons, the conduction rate is slower than would be predicted from the sizes of myelinated axons found in these nerves, and other properties characterizing this component such as chronaxie and refractory period set it off sharply from the previous groups *A* and B_1 (Heinbecker and Bishop, 1929). These properties appear to be more similar to those of the group to be described below than those of the other myelinated axons, and further data concerning them will be reported separately. Following the main *B* elevation, there appears, in the cat as in the turtle (figs. 2, 3, 6) a small wave whose threshold and conduction rate are closer to that of the B_2 than of the *C* process. It may be difficult to distinguish this small wave unless the record is diphasic, since it appears almost in the falling phase of the B_2 elevation. Also between this small wave and the succeeding large ones the potential may not return to the base line, indicating a distribution of fibers throughout this range without conspicuous maxima.

Finally with much stronger stimuli, one or more waves appear so much later that they cannot be recorded conveniently on the same time scale (fig. 6, *C* elevation). These waves occur in both vagus and sympathetic and are presumably due to unmyelinated fibers. The potential elevation assignable to these fibers is relatively greater in the vagus than in the sympathetic, corresponding to the greater number of unmyelinated axons in the vagus. There thus appear to be at least three types of fibers, possibly four, in these nerves, whose processes as measured by their action potentials, etc., can be differentiated. Group *A* consists of the subgroups of myelinated fibers which give rise to at least the alpha and beta waves, as in the frog sciatic, without insistence upon functional homology. Their essential differences are probably chiefly those depending on fiber size, and each is complex. With the *A* fibers might be included B_1 fibers with certain properties in common, or these might be considered as a separate group. Group B_2 consists apparently of myelinated fibers, and is in general distinctly double; its axons if myelinated have properties similar to unmyelinated axons. Group *C* presumably consists of unmyelinated axons, and is usually separable into several components, especially in the vagus. Group B_2 axons lie between the other groups in conduction rates and thresholds.

Other specimens of these nerves studied show distributions of potential closely similar to those pictured above, the significant differences being

TABLE 1

Thresholds and conduction rates of A, B and C potentials of autonomic and somatic nerve trunks

DATE	NERVE	TEMPERATURE	CONDUCTION DISTANCE	POTENTIAL	THRESHOLDS—ARBITRARY UNITS	CONDUCTION RATE—M.P.S.
			mm.			
January 1, 1929	Postganglionic turtle	21.7	21	B_2 1	2,500	4.4
				2	3,000	3.4
				3	3,000	
				4	5,300	2.6
				5	8,100	1.5
				C 1	12,000	0.9
				2	15,000	0.5
				3	25,000	0.3
October 10, 1928	Preganglionic turtle	21.7	12	B_1	400	8.9
				B_2	2,200	3.0
				C	5,400	0.9
December 3, 1928	Turtle vagus	22.5	33	B_1 1	400	15.6
				2	600	10.9
				3	600	9.8
				4	950	7.4
				B_2 1	2,400	4.6
				2	3,200	3.3
				B_3 1	5,000	1.3
				2		1.0
				C 1	9,000	0.9
				2		0.7
				3	18,000	0.5
December 26, 1928	Turtle sciatic	21.8	33.5	A	100	19.0
				B_2	2,500	3.0
				C	10,000	0.5
December 26, 1929	Bullfrog sciatic	25	66	A	100	59.0
				B_2	2,600	3.0
				C	20,000	0.7

Experiments of later date than those here reported indicate that in somatic nerves of the frog, turtle, cat and monkey there is a separable B_1 potential. Its threshold is approximately four to five times that of the most irritable fibers. Its conduction rate averages fourteen to eight meters per second in the frog and it has a short absolutely refractory period and chronaxie.

such varations of amplitude and conduction rate uniformly throughout the record as may be assigned to differences in vitality of the nerve as

a whole. The potential records are perhaps not as uniform as those of the sciatic nerve and the recorded potentials are lower These nerves are as rugged as other warmblooded nerves, and the unmyelinated fibers are as resistant to deterioration outside the body as the myelinated. Only rarely has any injury appeared assignable to dissection off of the connective tissue sheath.

IV. Thresholds and conduction rates. In table 1 are presented the thresholds of the main groups of action potentials of the vagus, sympathetic and sciatic nerves of the turtle, together with their subdivisions, the stimuli being short induction shocks. The threshold was taken as that stimulus which caused a distinct widening of the falling phase of the last wave and which was followed by a definite maximum rising from this falling phase. The investigation of more than fifty of such nerves indicates a relative constancy of ratios between thresholds, especially for the second and third groups.

The maxima occurring in the first group of these nerves are quite variable, especially in the sympathetic. Therefore, taking the threshold of the first potential as a standard gives discordant values for late potentials. If we take the threshold of the B_2 component as corresponding to that of the B_2 elevation of the turtle or frog sciatic, the A wave threshold in these nerves being taken as 100, the threshold of the first or B_1 process of the sympathetic would usually be about four hundred, and might be compared to the sciatic Delta wave. This is, however, arbitrary and we cannot draw definite analogies at present between fibers in the different nerves.

It is moreover not possible to compare directly the first group thresholds in the turtle with those in the cat. The cat vagus contains a group of thickly myelinated fibers of large diameter and low thresholds similar to the thickly myelinated fibers found in the peripheral nerves. The turtle vagus contains no similar group of fibers in the region studied, although according to Ranson (1915) a bundle of large myelinated fibers leaves the vagus in the cervical branch higher up. The waves due to the second group of B_2 fibers and of the C fibers in these nerves are much more constant. The threshold of the B_2 component is usually three to six times that of the B_1 component of the vagus or sympathetic. The first process of the C component usually has a threshold two to three times that of the B_2 component of the same nerve.

In table 1 are also presented typical conduction rates for the group and subgroup action potentials whose thresholds have been discussed above. In the vagus the conduction rate of the first process of the B_1 group varies from ten to sixteen meters per second, that of the B_2 from three and a half to four meters per second, that of the small third elevation of the B group 1.5 to 1 meter per second and that of the C group from 1.0 to 0.3 meter per second. The conduction rate of the main elevation of the potential

in the B_2 range is about three meters per second and in the C range about 0.8 meter per second. In the table the conduction rates of the action potentials of a typical turtle sciatic nerve are also given in comparison. It is seen that the conduction rates for the B_2 and C processes correspond to those of the B_2 and C processes in the vagus and sympathetic nerves.

Gasser and Erlanger (1926) have shown that there is a fairly definite relationship between fiber size (external diameter) and conduction rate particularly in the frog sciatic. Bishop and Heinbecker (1929) have presented data to indicate that there is an approximately linear relationship between threshold and conduction rate for the waves of the A group.

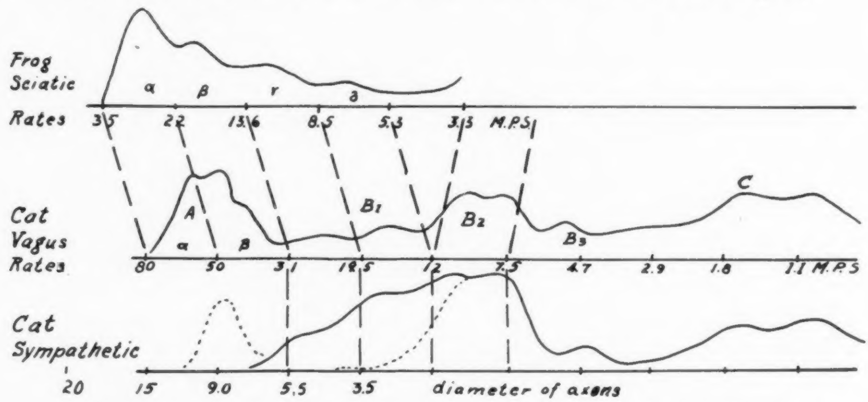

Fig. 7. Schematic diagram for comparison of potential records of sciatic, vagus and sympathetic nerves. The waves are plotted, for convenience, on a logarithmic time scale, and vertically, for the early parts of the records, the potentials along a perpendicular are due to axons of approximately the same size. Fiber sizes are plotted logarithmically. Actual amplitudes of potential in the sciatic are relatively much higher than drawn. The dotted lines indicate that in the cat sympathetic a first fast wave is occasionally present and also that the B_1 wave may be entirely absent, a condition so far found only when a separate depressor nerve is present.

A ten mu fiber in the turtle vagus on an average conducts fifteen meters per second. A fiber conducting at the rate of 3.5 meters per second would, if its rate varied as the diameter, have a diameter of 2.3 mu. However, the myelinated fibers of the turtle vagus are not less than 2.5 mu in diameter. If we were to consider threshold as approximately inversely proportional to conduction rate as it has been shown to be for the first myelinated fibers of the frog, the first fibers of the B_2 action potential of the vagus would be required to have a diameter of 3.3 to 2.5 mu and the fibers giving rise to the crest of the potential of this group would range from here down. Again it can be said that myelinated fibers of this diameter are never found in these nerves. The conduction rate of the B_2 component is always found

to be much slower than that which would be predicted from the actual size of the myelinated axons of these nerves, and the threshold is always higher. The conclusion may, therefore, be drawn that the fibers giving rise to the B_2 and C potentials do not fit into the scheme of properties characteristic of the A fibers of the somatic nerves of the frog and turtle while those giving rise to the B_1 potentials deviate less from the fiber size relation.

A similar conclusion has been arrived at with respect to conduction rate of the saphenous B elevation by Erlanger (1927) and with respect to the late potentials of the somatic nerves by Erlanger and Gasser (1930).

V. Histological findings. Having established the presence of four components of action potential the first arising from axons having the properties similar to those of the ordinarily thickly myelinated nerve fibers, the second from axons having properties slightly different from these and the others arising from axons very different properties, it is of interest to establish the origin of these groups of potentials. A method to accomplish this is found in a comparison of the cross section of sympathetic, vagus and somatic nerves.

The A component, not present in the turtle nerves in the regions studied but present in the cat vagus, is certainly assignable to large thickly myelinated axons present only in this nerve. It was stated above that in certain cervical sympathetic nerves of the turtle only two potential components were elicited while in others three appeared. In figure 8 C and D are shown cross sections of two such nerves. It will be readily seen that there are present, in both, unmyelinated nerve fibers and small thinly myelinated nerve fibers closely associated with the former. There are also present, in the second nerve, larger relatively thinly myelinated fibers which are entirely lacking in the first nerve. The first or B_1 potential of the sympathetic must then arise from the larger thinly myelinated fibers. In further confirmation of this we find that the cross section of the vagus nerve (fig. 8-E) which usually has a B_1 potential shows a histological picture similar to that of the second type of sympathetic nerve. When the vagus nerve does not exhibit a B_1 potential its cross section, also, lacks the large relatively thinly myelinated fibers then found in a separate nerve. The B_1 potential of the vagus can then likewise be considered to arise from larger thinly myelinated fibers. If, now, as seems reasonable, it can be assumed that the last potential elevation in these nerves arises from the unmyelinated nerve fibers it would follow that the next to the last potential component arises from the smaller thinly myelinated fibers common to both nerves. Further the potentials of one nerve (turtle vagus) have been recorded in which the B_2 component was, if not absent, at least not certainly detectable. This nerve was sectioned; its myelinated axons were mostly over 3.5 mu in diameter with a few below this figure,

while the unmyelinated axons were very numerous. The relative number of large and small axons in this nerve again corresponded to the relative prominence of the potentials observed and further corroberates the inference that the second group of potentials arises in small thinly myelinated axons. Finally, in a branch of the coeliac plexus of the cat (to be reported separately) a very large C elevation occurs with almost nothing else, and cross sections show a correspondingly small proportion of myelinated axons. Examination of the cross section of the turtle sciatic (fig. 8-F) shows the presence of larger and smaller thinly myelinated fibers, purely unmyelinated fibers and also larger and smaller thickly myelinated fibers. Its action potential exhibits three main groups the last two of which correspond in conduction rate to the B_2 and C components of the vagus and sympathetic nerves. The turtle sciatic is thus similar to the frog sciatic (reported by Erlanger and Gasser, 1929). The absolutely refractory periods of the B_2 and C components of sciatic nerves are similar to those of the B_2 and C components of the turtle sympathetic and vagus nerves here studied.

To summarize it can be said that these nerves, sympathetic with and without large fibers, and vagus, serve to differentiate between certain types of axons both anatomically and physiologically. All contain small thinly myelinated axons which by reason of the absence of larger fibers in the first sympathetic type can be designated as the source of the B_2 potentials of these nerves. The latter two contain larger thinly myelinated axons which in the second type of sympathetic and in the vagus give rise to the B_1 potentials, and in the sciatic possibly contribute to the late A potentials of this nerve. The sciatic nerve contains, besides the large and small thinly myelinated fibers, those which are thickly myelinated and which contribute to the more rapidly conducting waves present only in this nerve.

Further studies of the physiological properties differentiating these fibers types in more specific detail are being published separately; it may

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Fig. 8. *A*. Photomicrograph of cervical sympathetic of turtle. Action potential of this nerve illustrated in figure 3, film I; only group B_2 and C potentials obtained from this nerve.

B. Photomicrograph of cervical sympathetic nerve of turtle the action potential of which is illustrated in figure 3, film II; all three B_1, B_2 and C group potentials obtained from this nerve.

C and *D*. Photomicrographs of higher magnification of nerves illustrated in *A* and *B* respectively.

E. Photomicrograph of turtle vagus nerve, resembling second sympathetic.

F. Photomicrograph of turtle sciatic nerve. Magnification of *A* and *B* (printed lighter than *C*, *D*, or *E*) equal; magnification of *C*, *D*, *E*, and *F* $\times$ 1000; for general description of cross sections see text. Fixation and subsequent treatment of all nerves approximately alike osmic acid 1 per cent for 24 hours, paraffin sections, 5 mu thick.

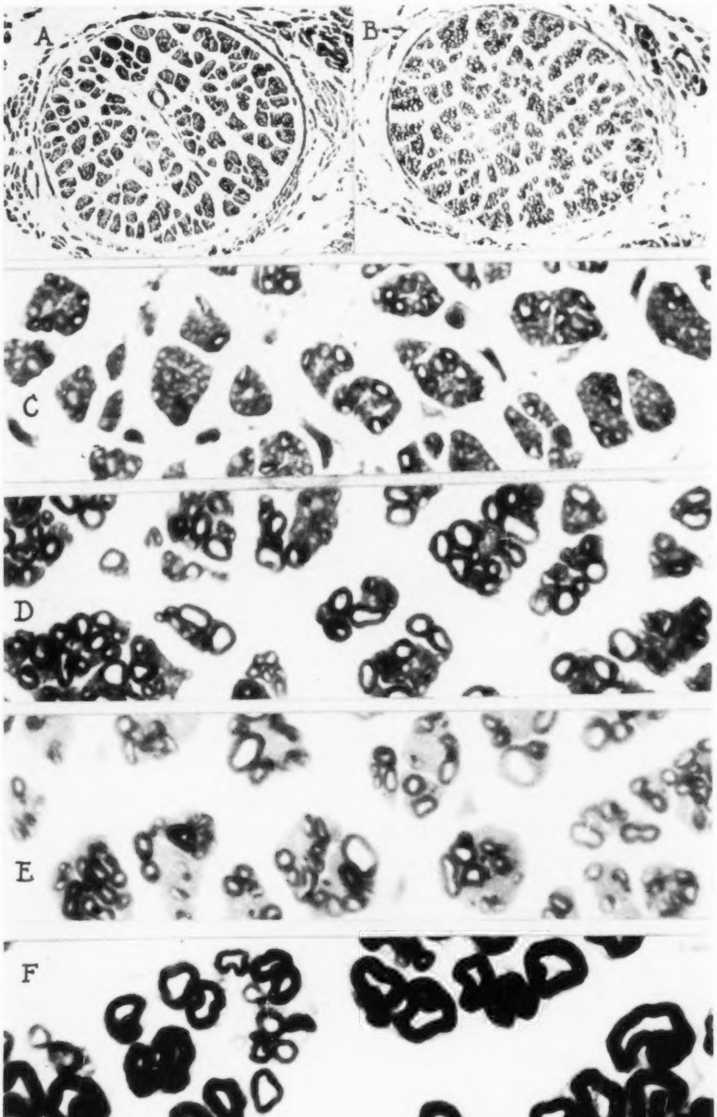

Fig. 8

be repeated here, however as stated above, that this differentiation is not complete or final; it is not excluded either that other types of axons than those recognized may contribute to the potentials recorded, or that other potential components will be found. In particular Erlanger and Gasser (1930) have further differentiated between components of the *C* elevation in somatic nerves and roots.

The histological sections indicate that the axon diameter of the larger unmyelinated fibers is often greater than that of some of the thinly myelinated fibers. Of axons of the same diameter those with myelin sheaths appear to conduct more rapidly than those without. While this relation-

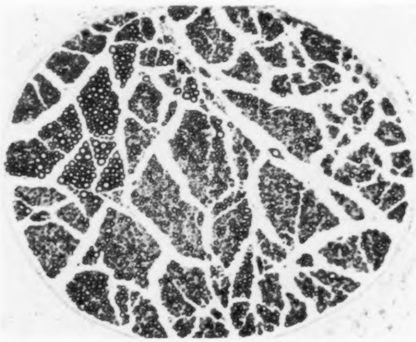

Fig. 9. Photomicrograph of vagus of cat, showing in upper part of the section groups of large fibers presumably responsible for the first double wave of the vagus record. The sympathetic cross section is similar except for the absence of these bundles of large fibers. Except for these, the fibers in both nerves grade from about 6 mu down. The largest axons are 15 to 17 mu. in diameter including the myelin sheath.

ship cannot be shown to be a causal one, the possibility exists that the myelination of an axon increases its conduction rate.

The histological picture of the cat vagus nerve stained with osmic acid (fig. 9) shows the typical myelinated fibers with relatively thick black sheaths, running in diameter down to 2.5 or 3 mu. Besides these, there occur other obviously myelinated fibers which have a diameter of 4 mu and less, but whose sheathes are definitely thinner and stain brown or gray in osmic acid (1 per cent for 24 hours).

Such thinly myelinated axons have been noted several times previously. Donaldson and Hoke (1905) in comparing the relation between area of axon and of sheath in cross sections of osmicated nerves of various animals, inferred that the thin and light staining sheaths indicated axons that were immature, but remarked that sheath thickness even of the more

densely myelinated axons varied more in small fibers than in large. Kiss and Mihalik (1928) describe fibers with thin sheaths in various animals, also fibers with intermediate thickness of myelin, designating them all as myelinated fibers. It is not clear from our observations in the cat where the line of demarcation lies, if any, between the ordinary thickly myelinated, and the thinly myelinated axons whose properties resemble those of unmyelinated. It is possible that no sharp demarcation exists, but, that the apparent distinctness of the potential processes results from the predominance of one type over the other in a range of sizes where a given conduction rate obtains, and, where, therefore, a wave appears prominently enough to be studied as an apparently distinct entity. Again it may be emphasized that what we see in the potential record are only maxima in a more or less continuous distribution, in which the predominant type of fiber characterizes the wave, without necessarily excluding other types of fibers which have the same rates of conduction; although as stated above the types of fibers can be more sharply distinguished by other properties, such as chronaxie and refractory phase (Heinbecker and Bishop, 1929).

A cursory inspection of the vagus cross section of the cat (fig. 9) identifies the first two or three waves of this nerve shown in figure 5, record I, as due to an area of large heavily myelinated fibers (10–15 mu) segregated in one region.

The rest of the smaller myelinated axons thus presumably give rise to the slower B_1 and B_2 potentials; except for the one region of thickly myelinated axons, the sympathetic cross section is similar to that of the vagus. The unmyelinated axons, although numerous in the section, do not show at this magnification.

Discussion. We have satisfied ourselves by technique previously described (Bishop, Erlanger and Gasser, 1926, and Heinbecker, 1928) that the potentials here discussed are simple responses to single stimuli, that is, they are neither repetitive responses, nor double responses from both anode and cathode of the stimulating circuit. If the nerve is killed only at the very end, the killed-live margin being close to the distal lead, the more rapidly conducting processes may appear to be monophasic (A) the slower ones diphasic (B and C). This is the result of the time required to conduct from the proximal lead to the killed end. All records are really diphasic, the second phase being depressed (Bishop, Erlanger and Gasser, 1926) but if the conduction time is short the first phase, being the most prominent, overlaps the second and extinguishes it in the record, while the slower the conduction the more distinctly diphasic the wave appears. Small elevations close to larger ones tend to fuse in monophasic records. The diphasicity of the B_2 wave in figure 6, record 7, is compensated by the first wave of the C process in figure 6, record 8.

The interpretation of the late action potentials is not always free of difficulty. Attention is directed to figure 2, films III and IV. In film III there is an unusually definite crest following the main crest of the C action potential which is not at all definite in film IV, when the stimulating current is of longer duration. This difference could be the result of the elimination of an anodal response by the current of longer duration stimulating all the fibers under the cathode (Heinbecker, 1928, loc. cit.). Likewise in figure 2, films VII and VIII, we have an atypical form of action potential arising on increasing the strength of stimulus. It will be seen that in VIII there is added a process earlier than the main process pictured in VII and also a much later process. These most probably represent repetitive responses of the earlier processes arising during the flow of a galvanic stimulus.

Attention is directed to the necessity of stimulating slowly (1 per second) and even the advisability of observing single potential deflections in the investigation of these fibers. Due to the long absolutely and relatively refractory phases of certain of the fiber groups, stimulation more frequently than once per second results in a reduction in total amplitude of the compound action potential and an alteration in the relative amplitudes of its potential groups. Rapid stimulation has been found to increase the duration of the absolutely refractory period.

The data here presented differentiate four components of potential on the basis of conduction rate and threshold, with such anatomical correlations as can be inferred from the correspondence between histology and potential in different nerves. Evidence has been presented in preliminary form (Heinbecker and Bishop, 1929) that further differentiation can be made on the basis of other properties, particularly duration of the absolutely refractory phase, chronaxie and form of the action potential at the stimulus. With respect to these properties, the fibers typical of the B_2 and C groups of these nerves appear to have much in common in spite of the apparent difference in myelination; and both differ from the fibers typical of the A and B_1 processes more than they do from each other. This is natural if the latter two groups of axons belong to the autonomic system, the functions of whose fibers are known to be slower in general than are those, for instance, of fibers to skeletal muscle.

Thus, although at present it cannot be said that parasympathetic and sympathetic groups of axons can be distinguished from each other, they can be distinguished from the ordinary somatic fibers. Somewhere between the afferent fibers and the autonomic efferent involved in visceral reflexes must occur the changes in properties from those of the A or B_1 processes to those of the B_2 and C processes, i.e., from the sensory to the motor side of the arc. This does not occur apparently in the cervical sympathetic ganglia, where the motor fibers all synapse with unidirectional

conduction, and sensory fibers may be absent from the nerve, and the properties of a given axon do not change at the synapse. (Heinbecker, experiments to be published.)

SUMMARY

An analysis of the action potentials of the vagus and cervical sympathetic nerves of the turtle and of the cat is presented.

In certain of these nerves there are typically four action potential components designated as the A, B_1, B_2 and C elevations. In others only the B_1, B_2 and C components are present and in still others only the B_2 and C, without, however, any prejudice as to homologies of function in different nerves with similar potential forms. Each of these main components also exhibits secondary potential maxima.

Threshold and conduction rates of the potential groups indicate the essential differences between the four potential components recognized. These together with a knowledge of their other properties such as form of potential at the stimulus, chronaxie and absolutely refractory period establish differences in their axon types.

Certain cervical sympathetic nerve trunks in the turtle exhibit only the B_2 and C potentials. A comparison of the histological picture of this type with that presenting three potential components B_1, B_2 and C affords a basis for the assignment of different nerve fiber types as origins of the various potential groups in the nerves studied. Evidence is presented to indicate that the larger thinly myelinated axons are typically those giving rise to the B_1 component of potential; that the smaller thinly myelinated axons contribute to the B_2 potential; and that unmyelinated axons are typically the source of the C elevations in these nerves. The A elevation when present is assignable to the larger thickly myelinated axons.

The B_2 and C components of these nerves contain the autonomic motor fibers. The B_1 component includes visceral afferent fibers.

I desire to acknowledge the interest of Dr. George H. Bishop in this work. I take this opportunity to express my appreciation of his assistance and direction in my research work in nerve physiology.[5]

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[5] We are indebted to Dr. E. V. Cowdry and his associates in the Department of Cytology for coöperation in the cytological phases of this research and especially to Mr. William Masek for the careful preparation of the sections. Dr. Otto Schwarz of the Department of Obstetrics has kindly put the factility of his photomicrographic laboratory at our disposal.

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THE EFFECT OF THE EXCISION OF DIFFERENT SEXUAL ORGANS ON THE DEVELOPMENT, GROWTH AND LONGEVITY OF THE ALBINO RAT[1]

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The purpose of this experiment was to study the effect of the extirpation of various sexual organs on the development, growth, general behavior, and longevity of the albino rat. All the animals were kept in the same room and were fed throughout the life span on the same carefully prepared synthetic food. Differences in results are therefore attributable to the character of the operation.

The operations performed on the male were gonadectomy and vasectomy. On the female they consisted of ovariectomy and hysterectomy. In vasectomy a portion of the vas deferens was removed and the cut ends ligated. In both operations on the male the incisions were sutured and the wounds dressed with collodion. We have previously described the operation for ovariectomy (Slonaker, 1927a). The operation for hysterectomy consisted of excising a part of the Fallopian tube and a considerable portion of the attached uterus leaving a stump of the cervical part of the uterus intact. This permitted both cut ends to heal over and thus prevent the passage of ova to the vagina. A rapid recovery without infection followed each of these operations and the disturbing effect rarely exceeded a day. Autopsies showed that all the operations were successful. All the operations were performed on immature animals at an age usually before sexual maturity. All the 8 males which were castrated were 44 days of age. These were selected from two separate litters. The 12 males which were vasectomized averaged 46.5 days at operation and were taken from three litters ranging from 46 to 48 days of age. In both groups of operated males the testes had descended previous to the operation. The 37 females which were ovariectomized were chosen from 10 litters whose ages ranged from 21 to 37 days and averaged 27.5 days at operation. These were all sexually immature. Of the 60 females which were hysterectomized 38 were selected from 12 litters whose ages ranged between 19 and 42 days and averaged 29

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the National Research Council through its Committee for Research in Problems of Sex.

days at operation. None of these had reached the age of sexual maturity as indicated by the vaginal orifice. The remaining 22 of this group were taken from 8 litters whose ages varied from 48 to 55 days and averaged 53 days when operated. In each of these 22 animals the vagina had opened previous to the operation. This indicates that these animals were very near to sexual maturity, but it is not known whether ovulation had occurred.

Opening of the vagina. According to Long and Evans (1922), the first oestrus in the rat was coincident with the opening of the vagina in 46 per cent of the 193 rats observed. In 22 per cent it occurred within five days after the opening and in 11.5 per cent it was delayed from 6 to 10 days. They give the average age at the opening of the vagina as 72 days with a range of from 34 to 109 days. They also state "In fact, in animals in which the ovaries have been ablated at about the thirtieth day of life the vaginal orifice is established at about the usual time, so that this event need not stand in any relation to the first oestrus." In our colony we have found that in the majority of normal healthy females the vagina usually opens before the age of 50 days. In the 39 females which were hysterectomized before sexual maturity the vagina opened at the average age of 46 ± 0.88 days and ranged between 39 and 68 days. From this we may conclude that hysterectomy does not influence the age at which an animal attains sexual maturity as indicated by the vaginal orifice. As we have previously shown (Slonaker, 1929) this operation does not interfere with the normal oestrual rhythm of the ovaries. In the ovariectomized animals we found a great variation and a wide range in the age at which the vaginal membrane disappeared. The earliest age was 44 days and the latest age was 177 days. However, 70 per cent of the cases were found to have occurred between the ages of 70 days and 115 days. The average for the group was 92 ± 3.65 days. In the two or three which showed almost normal development there may have remained a fragment of an ovary which was not extirpated. In many cases the vaginal orifice was very minute and seldom showed the condition found in normal and hysterectomized animals. The results of most of the animals of this group show that when ovariectomy is performed on sexually immature rats it is usually followed by a marked delay in the development of such sexual characteristics as the disapppearance of the vaginal membrane. The female gonads, therefore, in some way exert a decided influence in causing this phenomenon to occur at an earlier age. That some other influence is also involved is indicated by the fact that the membrane does later disappear in the absence of the ovaries. The results of Smith (1926) suggest that the pituitary gland may be responsible for this phenomenon which under the influence of the ovarian hormone is markedly speeded up.

Activity. The spontaneous running activity of each of these groups was recorded by means of our revolving cages which have been previously

described (Slonaker, 1908). The activity at approximately the same age (200 days) was used in making these comparisons. All animals had been in the revolving cages a sufficient time to have become completely accustomed to turning them.

Five normal male rats over a period of 10 days averaged 12798 revolutions daily. Five vasectomized animals for a similar time averaged 760 revolutions daily. Five castrated rats for a period of 10 days averaged 179 revolutions daily. Some of this last group were very lethargic and often did not turn the cage more than one or two revolutions during an entire day. This indicates that the male gonads in some way exert a stimulating effect on the animal which results in increased spontaneous activity. We do not know whether there is an escape of sperm or seminal fluid in the normal male rat deprived of coitus. The natural outlet for the testicular secretion is open and such a possibility may occur. In the vasectomized animals this free exit for secretions of the testes is blocked and there may thus result a harmful back pressure on the gonads and intervening structures, which causes them to function less actively in stimulating spontaneous activity.

In the females we found quite similar results. The average daily run for a group of 13 normal females was 11068 revolutions. The hysterectomized group averaged 8786 revolutions daily. In the ovariectomized group the average daily run was but 123 revolutions. These results indicate that the female gonads, like those of the male, exert a stimulating effect on the animal which results in increased spontaneous activity. This has been previously demonstrated. (Wang, Richter and Guttmacher, 1925; Hoskins, 1925; Slonaker, 1924, 1927.) Similar conditions are found in these three groups of females as existed in the three groups of males. In the normal female the egress to the exterior of the unfertilized ova and such excretions as may be present is unobstructed and the useless and disintergrating material may be voided without detriment to the animal. In the hysterectomized animals the only way in which these products can be gotten rid of is by resorption. It is possible that the resorption of the disintegrating ova acts as an inhibitor or toxic poison to the ovaries as well as other organs of the animal thus affecting their normal action. Abnormal pressure on the ovary may also exert an inhibitory effect. In the rat a tough transparent capsule, the bursa ovarica, completely incloses the ovary. During ovulation the periovarial fluid and the contained ova are put under an unnatural pressure by the contraction of the bursa ovarica because the natural outlet has been blocked by the healing over of the cut distal end of the Fallopian tube. The increase in pressure is also accentuated by the congested condition of the ovary at ovulation.

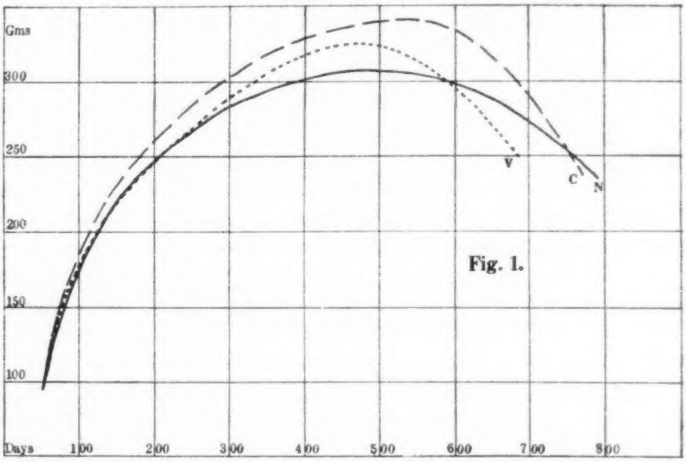
Sexual behavior. Vasectomy at an early age did not appear to influence the sexual activities of our rats in any noticeable manner. As soon as they became sexually mature they showed the same sex instincts and activities

as those exhibited by normal males. In order to determine if this operation had any effect when performed on fully grown males we vasectomized a few adults. No reduction in sexual desires or activities was produced in those operated after maturity. In all our males which were gonadectomized before sexual maturity no case of attempted coitus was observed. They did not manifest much interest in their surroundings. When placed with the opposite sex they exhibited about the same curiosity as that shown to a stranger of the same sex. To test the effect of this operation on mature animals two males at the age of 270 days and two others at the age of 330 days were castrated. The operations of the first two were performed in the morning. The evening of the same day they successfully copulated with receptive females as indicated by sperm in the vaginal smears. These continued to copulate daily for 25 days, but in no case was sperm found in the vaginal smears after the first coitus. Those operated at 330 days copulated for 28 days after operation. In all cases the sexual desires and activities became gradually less until they ceased on the days specified.

As previously stated, the hysterectomy operation did not influence the regular oestrual cycles. This group of females showed normal sex instincts and activities. At oestrus coitus occurred as readily as in normal females. Their activity curves showed regular oestrual peaks similar to those of normal females. Vaginal smears showed regular normal oestrual changes. The ovariectomized group exhibited a complete lack of sex instincts and activity. There was no manifestation of oestrus either in the vaginal smears or in their activity curves. The vaginal smears showed a condition of continued dioestrus.

Growth. In figure 1 we have given the average weight curves passing through the average maximum weight and terminating in the average death weight at the average age of death of the three groups of males. This shows that the group of gonadectomized animals very soon surpassed the weight of either of the other groups, and maintained a heavier weight for practically the whole of the life span. It is also seen that the vasectomized and normal groups exhibit almost the same curve of growth up to approximately 250 days of age. After this age the vasectomized group surpassed the normal for about 350 days and then the normal group became heavier. It is interesting to note, however, that the two or three vasectomized animals which lived the longest were heavier than the few normal males which lived to the greatest age in their group. In table 1 we have given the average maximum and death weights and the average ages at which they occurred in each group. This shows that the vasectomized group reached its maximum weight of 325 grams at the earliest age (475 days) and that the castrated group did not reach their maximum weight of 341 grams until the age of 549 days. The age at which the normal group attained the maximum weight of 307 grams was about midway between that of the other two groups.

Figure 2 shows the average weight curves of the groups of females. The more rapid increase in growth and the greatest maximum weight at-



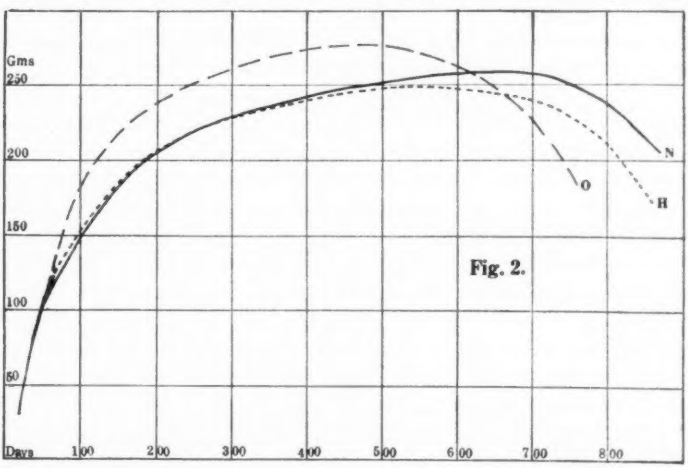


FIG. 1. Average growth curves of males showing average maximum and death weights. *N*, normal; *V*, vasectomized; *C*, castrated.

Fig. 2. Average growth curves of females showing average maximum and death weights. *N*, normal; *H*, hysterectomized; *O*, ovariectomized.

tained by the ovariectomized group is readily seen. This substantiates the results of Stotsenburg (1913). It is also noted that the average weights

of the hysterectomized and normal groups were very similar throughout the greater part of their lives and that the widest divergence occurred late in life. Table 1 gives the average maximum weights and the ages at which they were attained in these groups.

TABLE 1

Average maximum and death weights with average age of each

GROUP	NUMBER	MAXIMUM						DEATH					
		Age			Weight			Age			Weight		
		Range	Av.	P.E.	Range	Av.	P.E.	Range	Av.	P.E.	Range	Av.	P.E.
Males:													
Normal	10	296–684	504	±29.21	272–338	307	±4.93	660–1011	788	±22.25	156–308	237	±6.97
Vasectomized	12	276–732	475	±27.72	251–364	325	±7.46	276–958	685	±39.72	182–302	251	±7.88
Castrated	8	309–737	549	±4.56	291–420	341	±11.11	600–913	770	±28.00	160–310	236	±10.00
Females:													
Normal	17	394–910	705	±24.15	210–280	259	±4.71	466–1042	863	±27.69	145–266	209	±10.25
Hysterectomized	60	236–909	578	±15.56	198–342	250	±3.44	432–1168	855	±12.67	108–264	173	±4.19
Ovariectomized	37	97–825	483	±18.72	166–386	278	±5.66	163–1158	755	±22.15	120–300	185	±4.52

TABLE 2

Showing the average daily spontaneous activity in miles, the average daily food intake in calories and the average daily expenditure of energy of the different groups at the average age of 200 days

GROUPS	NUMBER	BODY WEIGHT	DAILY RUN	DAILY FOOD INTAKE	DAILY ENERGY EXPENDITURE			
					Loss in feces	Activity	Growth and basal metabolism	
		grams	*miles*	*calories*	*calories*	*calories*	*calories*	*per cent*
Males:								
Normal	5	245	10.89	101.28	15.19	58.19	27.90	27.5
Vasectomized	5	245	0.64	54.70	8.20	3.42	43.08	78.9
Castrated	5	260	0.15	55.40	8.31	0.85	46.24	83.3
Females:								
Normal	13	210	9.33	74.50	11.17	42.73	20.60	26.9
Hysterectomized	10	210	7.49	67.90	10.18	34.30	23.42	34.5
Ovariectomized	10	240	0.10	45.20	6.78	0.42	38.00	84.2

We thus see that the group in each sex which was deprived of gonads surpassed in growth and maximum weight either of the other groups of similar sex. Is an explanation at hand?

A study of the income and expenditure of energy will throw some light on the underlying cause for the differences found. We have previously

stated (Slonaker, 1927b) that one gram of our synthetic food contained 3.95 calories of energy. We have also ascertained (Slonaker, 1927c) that the energy used by the rat in running one mile was 0.02181 calorie per gram of body weight. Making use of these data we were able to tabulate the energy intake and expenditure for those animals of each group which were tested for spontaneous activity. A comparison has been made at the average age of 200 days and the results given in table 2. This table shows that though the food consumption was greatest in the normal groups of each sex their activity was also greatest and they used practically half the energy in this manner. There was thus left but a little more than 25 per cent of energy for growth and basal metabolism. In the males the fairly close approach of the growth curve of the vasectomized group to that of the castrated group may be explained by the large amount of available energy for growth in each of these two groups. The castrates grew the largest because they had the greatest amount of energy which could be used for growth. Figure 2 shows that the hysterectomized group slightly exceeded that of the normal females for the first 250 days. This can be explained by the fact that they had a greater amount of available energy for growth than was found in the normal group. This gives us the conditions of activity, food intake and energy expenditure as they existed at the age of 200 days. Our animals were not kept long enough in the revolving cages for us to secure data for similar comparisons at later ages. Judging from the curves of growth we are forced to conclude that the expenditure of energy changed as the animals grew older and that the per cent of energy intake which was used for basal metabolism and maintenance was not the same as found at the age of 200 days. We thus see that the greater the amount of spontaneous activity of an animal unless accompanied by a much increased food intake the less there will be of expendable energy available for growth. We think the causes for the differences in growth are intimately associated with and dependent upon the activity of the animal and the amount of energy intake.

In order to determine whether the greater weight of the gonadectomized groups over the other operated groups was due to an increase in stature, to a greater deposition of fat, or possibly both, measurements were made at autopsies of body and tail lengths. The average body length of 7 males which were vasectomized was 173 mm. (range 160 to 190) and the average length of the tail was 169 mm. (range 160 to 180). Similar measurements for the gonadectomized males were 167 (range 160 to 175) and 173 mm. (range 160 to 180) respectively. The ratio of body length to death weight in the vasectomized group was 1.45 and for the gonadectomized group it was 1.25. These results indicate that the surpassing in growth and maximum weight of the castrated males over the other groups of males was not due to a larger stature but most likely to a greater deposition of fat. It

appears that the testes in some way influence skeletal growth or that vasectomy stimulates it beyond normal. According to Stotsenburg (1909) castration does not modify the growth of the body in weight. Hatai (1913) also states "that not only the growth of the body in weight remains unmodified, but the relation between body length and body weight characteristic for this series is unaffected by castration." His observations were made on relatively young animals, the oldest being 284 days of age. The few measurements at death of normal males which we secured agree closely with those of the castrated group. The number, however, is too small to be conclusive. It would therefore seem that when young animals are vasectomized a condition is produced which tends toward greater skele-

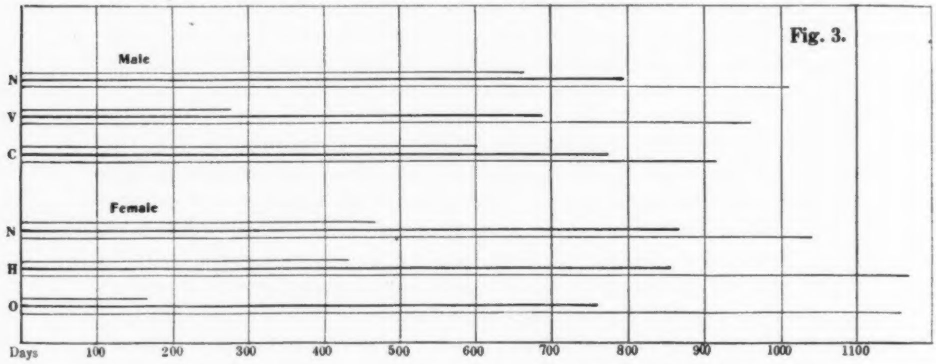


Fig. 3. Showing the minimum and maximum life span in light lines and the average span in heavy lines of the different groups. *N*, normal; *V*, vasectomized; *C*, castrated; *H*, hysterectomized; *O*, ovariectomized.

tal growth. This may be due to certain cell changes in the pars anterior of the hypophysis reported by Addis (1917) in castrated males.

In comparing the average body length at death of the females which were hysterectomized with that of the ovariectomized animals we find that the former had a body length of 167 mm. (range 145 to 185) and a tail length of 162 mm. (range 110 to 185) while in the latter the body length was 172 mm. (range 150 to 200) and the tail length was 167 mm. (range 150 to 190). The ratio of body length to death weight in each of these two groups was 1.036 and 1.075 respectively. These ratios are considerably lower than was shown in the male groups which indicates that the males were in relatively better physical condition at death than the females. The better growth and greater maximum weight of the ovariectomized group is thus apparently at least partly due to a greater stature, but as Stotsenburg (1913) has shown, it is also due to a greater deposition of fat.

Life span. In figure 3 we have indicated the average life span in heavy lines and the shortest and longest in light lines for each of the groups of animals. The probable errors of these averages are given in table 1. It is readily seen that the normal controls in each sex had the longest average life span. In the males, however, the normals exceeded the castrated group by only 18 days. The vasectomized group had not only the shortest average life span but also the greatest range in age at death of any of the males. From this it appears that the operation for vasectomy is more detrimental to longevity than that of castration. The slight difference, which was less than the probable error, in the life spans of the normal and castrated groups indicates that castration has little or no effect on the length of life in the rat. Not only was the greatest average life span found in the normal male group but also the individual which lived the longest belonged to this group. Figure 3 further shows that the life span of both the normal and hysterectomized groups of females surpassed that of any of the males. The normals exceeded the hysterectomized animals by only 8 days and the ovariectomized group by 108 days. Each of these groups of females had individuals which lived longer than any of the males. These results indicate that the extirpation of the ovaries early in life has a tendency to shorten the life span of the rat. A difference of less than the probable error in the life span of the normal and hysterectomized groups of females suggests that hysterectomy has little or no effect on longevity in the rat.

Cause of death. In as many cases as possible macroscopic post mortem examinations were made to determine, if possible, the cause of death. In some cases the dead were so mutilated by the cage mates that autopsies could not be made. The males of all the groups showed lung lesions. Pneumonia is a common disease in the rat at all ages, but especially so in senile rats. There was one doubtful case of death due to old age in a normal male which lived to the age of 1011 days. All other organs appeared normal. There were no cases of tumors, which often develop in senile rats, found in any of the groups of males.

In the females of all groups we found that lung lesions predominated as the cause of death. Other diagnoses were old age, intestinal inflammation and tumors. In the normal females 11.7 per cent had tumors, 17.6 per cent died of old age, and the rest lung infection. In many cases there were other complications as well as the lungs. The tumor-bearing rats all lived beyond 1000 days of age.

In the 45 autopsies made in the hysterectomized group all showed lung lesions. Seven of these were very old ranging in age from 1005 to 1168 days. The cause of death in these autopsies was complicated by intestinal disturbance in 4.5 per cent and by tumors in 13.3 of the cases. In most cases the tumors were associated with the mammary glands. They ranged in volume from the size of a walnut to 80 × 85 mm. In one rat

which weighed at death 220 grams the tumor was excised and weighed 100 grams. The youngest tumor-bearing rat to die was 817 days of age and the oldest which carried a tumor 30 × 33 mm. was 1168—the oldest rat in this group. In the whole group of 60 hysterectomized rats tumors were found in 10 per cent. These tumors often became infected, possibly due to some abrasion, and cancer-like.

In the ovariectomized group 13.5 per cent were considered as having died of old age complicated by lung trouble. In approximately 5.4 per cent lung lesions were associated with intestinal disturbance. Lung lesions were found in practically all cases. There were no cases of tumor found in this group.

It is significant to note that tumors were not found in any of the males nor the females which had been ovariectomized. That tumors were found only in animals with ovaries is very suggestive that these sex glands may be the cause of these abnormal growths. In a former experiment (Slonaker, 1928) we found in five different groups of females with ovaries intact that the per cent of those having tumors ranged from 7 to 50. In the five groups of males there was but one animal which had a tumor. Since the first appearance of tumors is closely associated with the menopause there is a close correlation between the cessation of functional activity of the ovaries and tumor development. Extirpation of the ovaries at this age might throw some light on the subject.

SUMMARY

When the operations in males for vasectomy and castration, and in the females for hysterectomy and ovariectomy were performed at an age before sexual maturity the following results occurred:

1. The vaginal membrane disappeared in hysterectomized rats at the average age of 46 days. This is approximately normal. In ovariectomized rats the average age was 92 days.

2. The average daily spontaneous activity in revolutions at the age of 200 days for the males was: Normals 12798; vasectomized, 760; castrated, 179. For the females: Normals 11068; hysterectomized, 8786; ovariectomized, 123.

3. The average daily food intake in calories at the age of 200 days for the males was: Normals, 101.28; vasectomized, 54.70; castrated, 55.40. For the females it was: Normals, 74.50; hysterectomized, 67.90; ovariectomized, 45.20.

4. The per cent of energy available for growth and basal metabolism at the age of 200 days for the males was: Normals, 27.5; vasectomized, 78.9; castrated, 83.3. For the females it was: Normals, 26.9; hysterectomized, 34.5; ovariectomized, 84.2.

5. The gonadectomized animals had the greatest maximum weight in

each sex. In the males this was due to a greater deposition of fat. In the females it was due to both a greater accumulation of fat and to an increase in body length.

6. The growth of the different groups is correlated with the amount of energy available for growth and basal metabolism.

7. Both sexes of gonadectomized animals showed a complete lack of sex instinct or activity. Normal conditions followed the other operations.

8. The vasectomized group had the greatest body length at death of any of the males. The ovariectomized group of females surpassed in body length the other groups of the same sex.

9. The average life span in days from longest to shortest in the males was: Normals, 788; castrated, 770; vasectomized, 685. For the females it was: Normals, 863; hysterectomized, 855; ovariectomized, 755.

10. Most of the deaths in the male groups were due to lung lesions. There was one death due possibly to old age. In the female groups lung infection was the principal cause of death. In some cases there were complications, such as old age, intestinal disturbance, and tumors.

11. Tumors were found in approximately 10 per cent in each of the normal and hysterectomized groups. They were not found in any of the other groups of either sex.

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THE EFFECT OF CARBON DIOXIDE ON NERVE[1]

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The older literature (Biedermann, 1898; see also Piotrowski, 1893) contains several striking statements concerning the action of carbon dioxide on nerve: Perhaps most interesting is the claim that a stretch of nerve exposed to carbon dioxide is able to conduct through it impulses initiated in normal nerve above it at a time when it is itself entirely inexcitable by applied electrical stimuli. If correct, this observation would place serious difficulties in the way of present theories of nerve conduction, which regard the potential changes at an active region as the means of stimulation of an adjacent resting one. Normal propagation thus depends on the excitation of each region by electric currents originating in active nerve; and so long as a stretch of nerve is able to conduct an impulse it should retain its irritability to electrical stimuli, whether arising within or without the nerve. The observation referred to is not in accord with this and would seem to show that the natural stimulation of resting by active regions is by some other mechanism. It appeared desirable, therefore, to re-examine the relations of "excitability" and "conductivity" under the influence of this gas. Further, interference with oxygen supply to a tissue is associated with changes in carbon dioxide content and it was desired to supplement the study of oxygen lack in a preceding paper (Gerard, 1930) with an examination under identical conditions of the carbon dioxide effect.

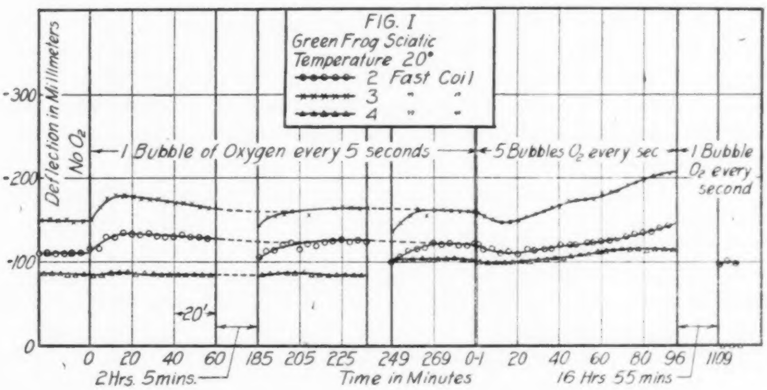
Several recent papers have dealt with this (Cooper, 1924; Davis, Pascual and Rice, 1928; Heinbecker, 1929; Amberson and Downing, 1929), but, in most, conditions were not the same as in the present work. The findings of Amberson and Downing (1929) and the classic ones of Waller (1897) were obtained with slow recording instruments, as in these experiments. Most workers agree that CO_2 in low concentration causes an increased action potential, due largely to prolongation.

Method. The conditions of experimentation were the same as those described in a preceding paper (Gerard, 1930). Sciatics of green frogs and bull frogs were used. The various mixtures of carbon dioxide and oxygen

[1] A preliminary report of this work appeared in the Proceedings of the Institute of Medicine of Chicago, 1929, vii, 202.

were made from cylinders of the pure gases and kept in large bottles over slightly acidulated water. Pure oxygen was kept slowly flowing through all partitions of the chamber except as a carbon dioxide mixture was substituted in the central partition. Care was taken to maintain a fairly uniform rate of gas flow (about one bubble per 3 sec.), for gross changes in rate somehow modified the electric responses obtained from the nerve. This was especially true with carbon dioxide, an increased rate of bubbling leading to an increase in responses.

For the experiments on conduction, using the action potential in the region of nerve exposed to carbon dioxide as the measure, the nerve chamber with multiple leads was used. Induction shocks at 304 and 88 per second were applied to a portion of the nerve remaining in oxygen, above or below the carbon dioxide stretch.

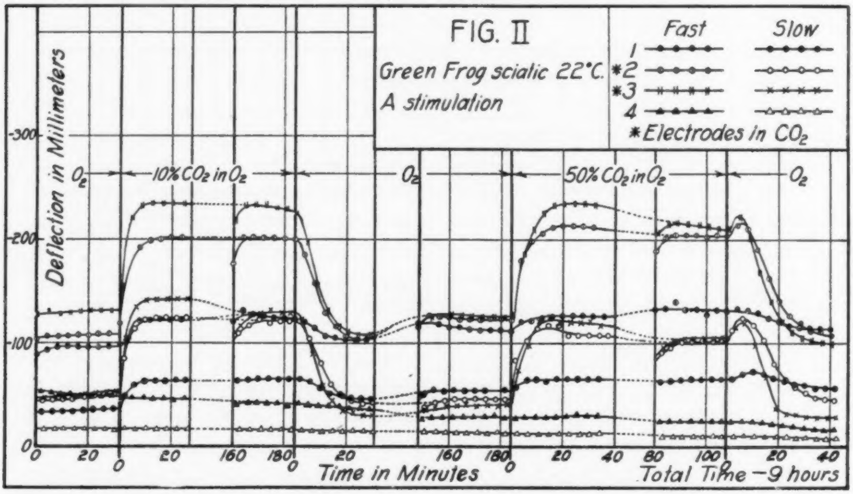


The same types of variation of action potentials were encountered as previously discussed, including especially changes with time since dissection and with intervals between stimulation. Figure 1 indicates the degree of constancy obtained when all conditions were carefully controlled.

The direct studies of excitability were made in a paraffin chamber using a muscle nerve preparation. One end partition contained the muscle, the middle and other end partition were each supplied with a pair of stimulating electrodes, on which the nerve lay. Carbon dioxide was passed through the middle partition. The far electrodes served to test for irritability changes at an unexposed part of the nerve and for conduction through the exposed stretch. The near electrodes, of course, tested irritability changes in nerve exposed to the carbon dioxide. Threshold was measured in three ways, at each electrode pair: 1. A single make or break shock was taken from a cored Harvard coil in the usual manner.

The primary current was kept constant (2 volts across the coil) and the secondary coil kept at 12 cm. (usually), the coil angle, measured on a protractor, being varied to alter the strength of stimulus. 2. A single make or break shock from a coreless Harvard coil was used, stimulus strength being controlled by a series resistance in the primary circuit. 3. The chronaxie and rheobase, as well as the voltage-time curve, were determined by Lapicque's condenser method (Lapicque, 1926). Threshold changes were not identical for these forms of stimulating current.

RESULTS. a. *Changes in action potentials of a nerve stretch exposed to carbon dioxide.* The immediate effect of admission of carbon dioxide is a marked increase of the total action potentials. This is true for all nerves



studied, for all concentrations of carbon dioxide used, 10 per cent to 100 per cent, and can also be observed in nerve that retains only a small fraction of its initial activity after long standing. The action potentials rise to values 200 to 400 per cent or more of the original ones, and the extent of the increase is quite independent of the concentration of the carbon dioxide, within the range studied (fig. 2, tables 1–2). This is at variance with the experience of Davis, Pascual and Rice (1928), and of others. They report a depression of responses with CO_2 concentrations of 10 per cent or more. After a maximal value is reached, it is maintained constant for long periods (3 hours or more) except in the case of 100 per cent CO_2. In pure CO_2 the maximum is held for a short time, after which responses fall very much as in nitrogen and are abolished in the same time as in

nitrogen, two to three hours. Since in 90 per cent CO_2 and 10 per cent O_2 no such fall occurs, it appears to be due to simple asphyxia, as when oxygen is replaced by any inert gas.

The increased response might be due to prolonged or to greater potentials, as discussed in the preceding paper; and again comparison of the results with fast and slow tetanization yields evidence of prolongation. The F/S ratio falls markedly; that is, the responses to slow tetanization increase proportionately much more than to fast (tables 1 and 2). Thus, in a typical experiment on a green frog sciatic, the F/S fell from 2.2 at the start to 1.6 at the maximum at the upper electrode (2) in CO_2 and from 2.6 to 1.6 at the lower one (3). The electrode below the CO_2 stretch remained nearly constant (slight fall), showing that most impulses were still able to traverse all the fibres in the exposed region. A slight fall in the F/S ratio was often observed, which indicates a prolongation of the refractory period.

b. *Changes in action potential of an exposed nerve stretch on replacing carbon dioxide by oxygen.* The prompt effect of replacing CO_2 or a CO_2-O_2 mixture in one partition by a stream of pure oxygen is a further increase in action potentials. They rise to a peak value, 5 to 15 per cent, above the maximum obtained in the CO_2 period, and then sharply fall to a new level which is maintained. This is usually less than the original one or than the level reached by nerves remaining in oxygen for a similar length of time, and indicates a permanent damaging effect of the CO_2. The initial peak is not always present, and is more likely to be absent following treatment with a low CO_2 concentration than with the stronger mixtures (fig. 2, tables 1 and 2). Since the peak often reaches values greater than attained in CO_2, it cannot be due to low CO_2 concentration during out diffusion, as suggested by Davis, Pascual and Rice.

The F/S ratio is lowered still further at the peak and then rapidly returns to or towards its original values.

c. *Time course of the changes.* The start of the increase of action potentials occurs very promptly after the admission of CO_2 (table 3). In over half the experiments on green frog, the response obtained at 45 seconds had risen, and in several the response even at 15 seconds was increased. In only two cases was the rise delayed beyond the third observation at 75 seconds, and in these it had appeared by 135 seconds. In general the effects of the CO_2 were first manifested between 30 and 60 seconds (average 42 sec.) after its admission. This time was independent of the concentration used, being no greater for 10 per cent than for 90 per cent CO_2 (though intermediate concentrations seemed to act less rapidly) and was the same at all electrodes showing a change—inside or above the CO_2 compartment. For bull frog nerve, though fully twice the radius, the first effect was seen as promptly, in 45 or 75 seconds, average 47 seconds. This "latent period" could hardly represent diffusion into

TABLE 1

Bull frog sciatic 20°C. February 9, 1929

CONDITION	LEAD 1			LEAD 2			LEAD 3*			LEAD 4*			LEAD 5		
	F	S	F/S	F	S	F/S	F	S	F/S	F	S	F/S	F	S	F/S
40 minutes in O_2	110	47	2.34	126	58	2.17	134	63	2.13	156	77	2.03	114	45	2.52
50 per cent CO_2 in O_2 in middle partition:															
Maximum (20 minutes)	203	126	1.62	230	150	1.53	327	222	1.47	362	253	1.43	109	42	2.59
At 30 minutes O_2 admitted:															
Peak (8 minutes)	212	142	1.48	239	164	1.46	344	250	1.38	374	275	1.36	(106)	43	2.46
Steady (70 minutes)	73	25	2.91	88	35	2.52	116	48	2.42	119	51	2.34	93	34	2.74
70 per cent CO_2 in O_2 in middle partition:															
Maximum (10 minutes)	193	122	1.58	217	140	1.55	326	218	1.43	370	258	1.43	88	36	2.44
At 25 minutes O_2 admitted:															
Peak (8 minutes)	—	123		—	152		327	243	1.35	—	268		—	(36)	
Steady (130 minutes)	68	27	2.52	78	35	2.23	102	50	2.04	98	45	2.17	79	28	2.83
90 per cent CO_2 in O_2 in middle partition:															
Maximum (15 minutes)	208	117	1.86	228	131	1.64	342	203	1.68	390	249	1.56	73	31	2.35
At 35 minutes O_2 admitted:															
Peak (8 minutes)	—	144		—	161		344	251	1.37	393	291	1.35	80	32	2.50
Falling, nearly steady (50 minutes)	64	22	2.90	75	30	2.50	111	52	2.14	99	41	2.41	80	30	2.66
20 hours later	110	62	1.78	115	61	1.88	126	66	1.91	144	70	2.06	57†	24†	2.38
15 hours later	117	50	2.34	114	50	2.28	108	49	2.10	99†	44†	2.15	0	0	
90 per cent CO_2 in O_2 in middle partition:															
Maximum (15 minutes)	118	70	1.68	116	73	1.59	116	82	1.42	18‡	44	0.41	0	0	
At 25 minutes O_2 admitted:															
Peak (8 minutes)	191	134	1.43	201	139	1.44	228	163	1.40	200	138	1.45	0	0	
Falling, nearly steady (40 minutes)§	117	45	2.60	106	44	2.41	102	45	2.31	88	35	2.52	0	0	

Values as fractions of initial:															
Maximum in 50 per cent CO_2	1.85	2.58		1.82	2.59		2.44	3.53		2.33	3.24		0.96	0.93	
Peak in O_2	1.92	3.02		1.90	2.83		2.56	3.96		2.40	3.57		0.93)	0.95	
Steady in O_2	0.66	0.53		0.70	0.60		0.87	0.76		0.76	0.61		0.81	0.75	
Maximum in 70 per cent CO_2	1.75	2.60		1.63	2.41		2.43	3.46		2.37	3.35		0.77	0.80	
Peak in O_2	—	2.62		—	2.62		2.44	3.85		—	3.49		—	0.80)	
Steady in O_2	0.62	0.57		0.62	0.60		0.76	0.79		0.63	0.59		0.69	0.62	
Maximum in 90 per cent CO_2	1.89	2.49		1.81	2.26		2.55	3.22		2.50	3.23		0.64	0.69	
Peak in O_2	—	3.06		—	2.77		2.56	3.98		2.52	3.78		0.70	0.71	
(Steady) in O_2	0.58	0.47		0.59	0.52		0.83	0.82		0.63	0.53		0.70	0.67	
20 hours later	1.00	1.32		0.91	1.05		0.94	1.04		0.86	0.91		0.50	0.53	
15 hours later (these values as new initial)	1.06	1.06		0.90	0.86		0.81	0.78		0.63	0.57		0.0	0.0	
Maximum in 90 per cent CO_2	1.01	1.40		1.02	1.46		1.08	1.67		0.18	1.00				
Peak in O_2	1.64	2.68		1.76	2.68		2.11	3.32		2.02	3.14				
(Steady) in O_2	1.00	0.90		0.93	0.88		0.95	0.92		0.89	0.80				

* Electrodes 3 and 4 exposed to CO_2.

† We have often observed, on isolated sciatics kept for long periods of time, that the more peripheral end fails first and inactivity progresses centralward. This more complete inactivity at the injured end may also be the cause for the increased responses obtained at more central leads (cf. Gerard, 1930).

‡ Note distal electrode in CO_2 falls and later recovers. This is one of the very few instances we have observed of depression of action potentials by CO_2.

§ The nerve was somewhat putrified when removed.

the nerve bundle, since varying size of nerve and varying CO_2 concentrations did not influence it. Nor should diffusion far into the interior of the bundle be necessary for a first effect to appear, since the more superficial fibers would respond earlier. Despite this consideration it seems improbable to us that the critical factor here is diffusion, even into individual fibres, for 1, the CO_2 concentration in any fibre should rise faster with greater external CO_2 pressure, and 2, the thicker sheath of the

TABLE 2

Green frog sciatic. Temperature = 23°C. January 28

CONDITION	LEAD 1			LEAD 2			LEAD 3			LEAD 4		
	F	S	F/S	F	S	F/S	F	S	F/S	F	S	F/S
2 hours rest in O_2	98	37	2.65	110	50	2.2	132	52	2.6	50	18	2.8
10 per cent CO_2 in O_2 in middle partition:												
12 minutes	123	63	1.95	201	125	1.6	238	144	1.65	48	18	2.7
180 minutes	124	65	1.90	201	121	1.65	233	131	1.8	41	17	2.4
At 185 minutes O_2 admitted:												
6 minutes	118	62	1.90	175	105	1.65	190	95	2.0	41	17	2.4
30 minutes	103	46	2.25	105	42	2.5	108	32	3.4	37	16	2.3
180 minutes	112	54	2.1	123	47	2.6	126	40	3.15	30	13	2.3
At 185 minutes 50 per cent CO_2 in middle partition:												
20 minutes	128	66	1.95	215	117	1.85	237	122	1.9	30	13	2.5
100 minutes	131	66	2.0	205	103	2.0	215	107	2.0	25	10	2.5
At 110 minutes O_2 admitted:												
6 minutes	130	70	1.85	216	125	1.7	225	120	1.85	24	10	2.4
30 minutes	113	55	2.05	108	44	2.45	110	28	3.9	19	8	2.3
Values as fractions of initial:												
Maximum in 10 per cent CO_2	1.25	1.75		1.8	2.5		1.8	2.8		0.95	0.95	
3 hours in O_2	1.15	1.45		1.1	0.95		0.95	0.8		0.8	0.7	
Maximum in 50 per cent CO_2	1.3	1.8		1.95	2.35		1.8	2.35		0.6	0.65	
Peak in O_2	1.3	1.9		1.95	2.5		1.8	2.3		0.5	0.55	
30 minutes in O_2	1.15	1.5		1.0	0.9		0.85	0.55		0.4	0.45	

bull frog nerve should have introduced a greater delay. It is more likely that HCO_3' reaches the outer nerve fibers well within the time observed and then requires a further interval to produce its action. A consideration of the time required for the full response to CO_2 supplies additional evidence for this view.

Although the first effect of CO_2 usually appears well within one minute, the maximum effect is not reached, on the average, for 20 minutes or more

(table 3). The time, though somewhat variable from one experiment to another, is the same for all electrodes responding and is independent of

TABLE 3

PER CENT CO_2	IN CARBON DIOXIDE								IN OXYGEN AFTER CARBON DIOXIDE									
	First change (seconds after CO_2 in)					Maximum (minutes after CO_2 in)			First change (seconds after O_2 in)							Maximum (minutes after O_2 in)		
	15	45	75	105	135				45	75	105	135	165	195	255			
	Lead 1	Lead 2*	Lead 3*	Lead 4	Lead 1	Above	Exposed	Below	Lead 2*	Lead 3*	Lead 4	Lead 1	Lead 2*	Lead 3*	Lead 4	Above	Exposed	Below
Green frog sciatic																		
100	x					12	11	2	x							—	—	—
100	x					20	23	—					x			14	15	—
10	x					10	7	—					x			6	9	—
10		x				16	17	—					x			10	11	8
10		x				18	15	—		x						12	13	—
90		x				42	49	2		x						10	11	—
† 10	x					10	13	—										
50			x			16	15	—					x			6	5	—
50			x			18	21	—					x			3	4	—
90		x				8	9	—					x			4	3	—
40			x			8	15	—	x							6	5	—
50		x				24	25	—					x			—	3	—
60		x					17	—					x			—	25	—
33		x				16	23	—				x				—	—	—
40					x	12	13	—				x				—	—	—
50			x									x				4	5	—
33		x				14	13	—				x				4	5	—
70			x			14	15	8					x			—	3	—
90		x				12	13	4				x				2	3	—
50					x	18	25	—					x			—	3	—
Bull frog sciatic																		
	Lead 1	Lead 2	Lead 3*	Lead 4*	Lead 5				Lead 2	Lead 3*	Lead 4*	Lead 5	Lead 1	Lead 2	Lead 3*			
50			x			16	14	—						x		6	7	—
70		x				11	11	—						x		8	9	—
90		x				21	17	5					x			8	9	—
90			x			11	14	—				x				6	7	—
90			x			9	14	—							x	10	11	—
70			x			7	11	5							x	10	11	11
50		x				10	11	—							x	10	8	—

* Leads in CO_2 partition.

† Experiments on one nerve.

CO_2 concentration or nerve radius. This interval between first and maximum effect must be due to a progressive increase in the number of fibres affected or to a progressively increasing response per fibre. Since CO_2 penetrates the sheath and acts on some fibres within a minute it could hardly require 20 minutes to penetrate to the center of the bundle, so that the major part of the rise must be due to increasing potentials per fibre. This, in turn, cannot depend on the slow increase in HCO_3' concentration at each fibre. If this were the case, since 10 per cent CO_2 in the gas leads to the same final maximum as 90 per cent CO_2, the maximum effective concentration should be reached earlier with the stronger CO_2 mixtures. The picture is rather that of a gradual change in the state of each nerve fibre in response to a rapid change of conditions. The presence of the CO_2 sets off a slow reaction. One is led to consider the influence of a diminished pH on some metabolic processes, especially in view of the effect of acidity on the refractory period of nerve (Adrian, 1920).

The time required for the nerve response to change after the CO_2 in its surrounding gas has been replaced by oxygen is also of interest. This has been surprisingly constant and, as for the admission of CO_2, is independent of the CO_2 concentration with which the nerve had come in equilibrium, and of the location of the electrode within or above the exposed stretch. The interval is longer than that following the admission of CO_2, averaging 2 minutes for green frog nerve and is still greater, 3 minutes, for bull frog nerve (table 3). Following this first change, the peak response in O_2 is reached in 3 to 15 minutes. Although too variable to average, it is apparent that the peak is attained and passed in considerably less time than that required for the maximal response in CO_2. This peak, however, cannot be regarded in any sense as an equilibrium and is hardly even a true maximum, as it may represent an interesection of two changes. The true equilibrium level, usually below the original one before CO_2 treatment, is not reached for a considerable time, 60 minutes, after the change to O_2.

It is worth noting that the first effect of adding CO_2 appears in one-third the time required for the first effect of removing it to appear. Similarly the maximum change in CO_2 is achieved three times as rapidly as the return on its removal.

CO_2 may well produce changes in the fibres at a certain rate which are not reversed as rapidly on its elimination. As in the case of the first effects, the time of return is independent of the CO_2 concentration and probably measures the rate of some reaction in the nerve fibres rather than the out diffusion of the gas. Adrian (1920) likewise observed long delays between exposure of a nerve to a solution at some new pH and the change in the nerve.

d. *Change of action potential at regions of the nerve not exposed to carbon dioxide.* Although CO_2 was admitted into only the central compartment

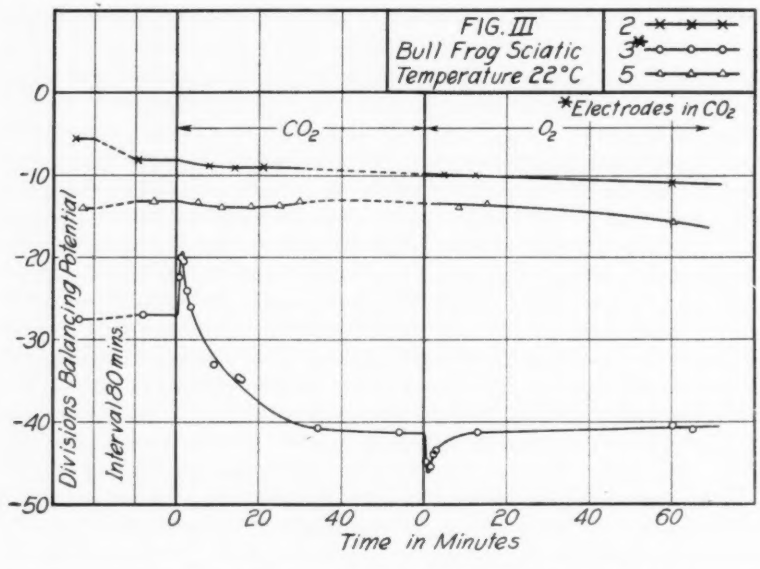
of the chamber, electrodes leading from other portions of the nerve as well showed changed responses. Electrode 1 (or 1 and 2) between the stimulated part (A) and the CO_2 stretch regularly paralleled the change shown by the electrodes in the CO_2 stretch to any gas mixture. Electrode 4 (or 5 and 6), beyond the CO_2 stretch, exhibited similar changes in 6 cases out of 25. Whatever changes appeared at these outside electrodes were in the same direction and followed the same time course as those in the exposed region. They were manifested just as promptly at electrodes 3 cm. beyond the middle compartment as at those in it, but were never as intense outside as in. Where two electrodes were used above the CO_2 stretch (1 cm. and 3 cm. away) both showed changes of the same magnitude and occurring at the same time.

Diffusion of CO_2 along the nerve to the outside regions cannot be the cause of their responses. It is difficult to conceive of CO_2 or HCO_3' diffusing several centimeters along a nerve when a lateral diffusion of half a millimeter or less would cause it to leave the nerve entirely and be washed away in an oxygen stream; and it is impossible to conceive its diffusing back again to the central compartment when the CO_2 in that is removed. The first might be interpreted in terms of greater diffusibility along the axone core than across the membrane or nerve sheath, but the second could not. Further, diffusion of CO_2 should bring about outside responses after some delay, more for the farther electrodes, should cause lesser changes at far than at near ones, and should affect equally those above and below the CO_2 stretch, since they are symmetrical to it. Actually none of these expectations are realized. In the preceding paper a similar participation of outside electrodes in the case of asphyxial changes was described, and the conditions of its appearance considered. We have no reason for supposing the present situation different, except for some responses at the lower electrodes. Since changes at electrodes below the CO_2 region were infrequent and slight when present, and especially since they were limited to the nearer one, 9 mm. from the partition, it is probable that these do represent the effect of slight diffusion along the nerve.

e. *Equilibration.* When a nerve in oxygen is tetanized at 300 shocks a second for one minute, the action potential (as recorded by the deflection of a critically damped galvanometer previously held at zero by a balancing potential) steadily increases during this time. This is a manifestation of the changes of equilibration, previously described. In carbon dioxide this effect is abolished, or even reversed. Thus during a 60 second tetanus in oxygen one nerve (bull frog sciatic, 21°C.) gave the following galvanometer readings at 10 second intervals: 0(start), −24, −25, −28, −32, −38, −42. Later, in CO_2, the following were obtained: 0(start), −160, −153, −150, −148, −147, −147. This might mean that, if equilibration be due to CO_2 accumulation, in pure CO_2 no further effect is possible.

Davis, Pascual and Rice (1928), using weaker carbon dioxide mixtures, also obtained no evidence of equilibration with carbon dioxide. The changes in single action potentials (see, for example, Gerard and Forbes, 1928) or summed responses, induced by repetition, would thus seem not to be due to carbon dioxide accumulation as the result of activity.

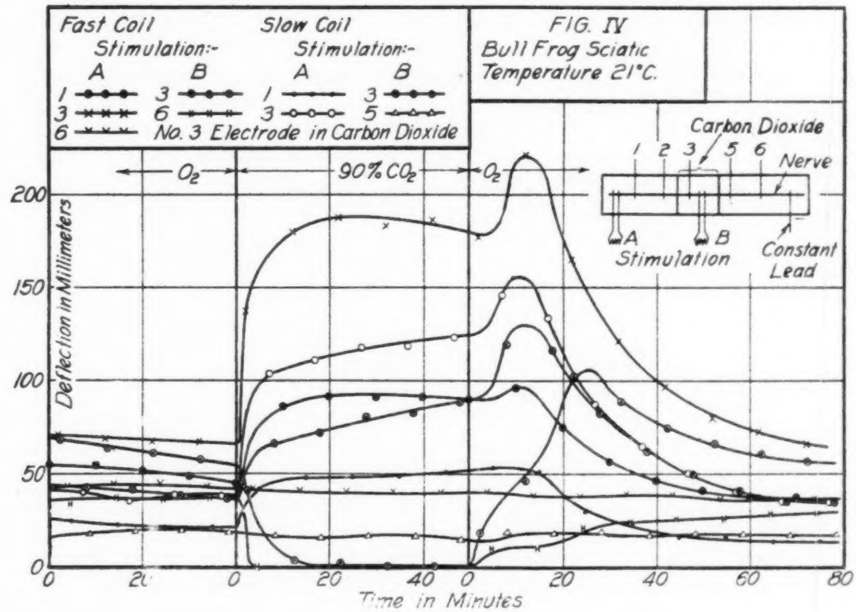
f. *Resting potentials.* The measurement of changes of the resting potentials has been complicated by the marked effects produced on changing the rate of bubbling a gas through the chamber. We have regularly observed that an increased rate of bubbling causes a decreased potential (side less positive), and vice versa. This occurs even when only one gas is being used and when this has been brought to room temperature



and saturated with moisture. When this factor is controlled the experiments all have yielded results qualitatively alike but showing great quantitative variation. The first change, on admitting CO_2, is an increase in potential, followed in a few minutes by a fall to values considerably below the original ones and lasting for a longer period (fig. 3). When the CO_2 is replaced by oxygen reverse changes occur, though the potential tends to remain lower than before the CO_2 exposure. It is significant that the action potentials are increased by the same agent that lowers the resting membrane potential. If the activity involves mainly a temporary depolarization of the existing potential, a decrease in this latter should involve a similar decrease in the potential change of activity. It is possible that the

amplitude of the potential changes is actually decreased (as Heinbecker (1929) reports—though the possibility exists, since he stimulated a region exposed to CO_2, that some of the fall observed was due to loss of irritability; see later), the total effect being greater because of very marked prolongation of the potentials. Certainly, when the nerve is again in oxygen after CO_2, both action and resting potentials are usually decreased.

That such prolongation does occur is evident from our results as well as those of Amberson and Downing (1929), Davis, Pascual and Rice (1928) and the older ones; of Boruttau (1901), for example. Heinbecker (1929),



using the Braun tube, does not find any evidence of prolongation, presumably because his records did not include the later changes following conduction. Such prolonged potentials must represent slowed changes during the recovery from conduction, as in an acid medium or in asphyxia. If a fall of the intensity of action potential does not accompany a fall of the resting membrane potential it would constitute evidence for their independence. The important question of whether the action potential represents 1, essentially a passive decrease in an existing membrane potential, due to breakdown of polarization, etc., or 2, an active development of a negative potential due to chemical activity has received hardly

any experimental answer. The careful correlation of changes in each under a variety of conditions may be expected to yield one. During asphyxia the two appear to run fairly parallel, though fibre block is reached before the changes in resting potential are complete.

g. *Changes in threshold in nerve exposed to carbon dioxide.* The influence of CO_2 on nerve threshold depends markedly on the type of currents with which it is measured. With the usual cored-coil used for stimulation in the CO_2 compartment and the nerve response measured by action potentials, relatively little change in the threshold resulted from the admission of CO_2. One experiment, however, gave a very striking result (fig. 4). Action potentials at all electrodes showed the usual changes on the admission of CO_2 when the stimuli were applied above the exposed region. With the slow coil, they also rose as usual for stimuli applied within the CO_2

TABLE 4

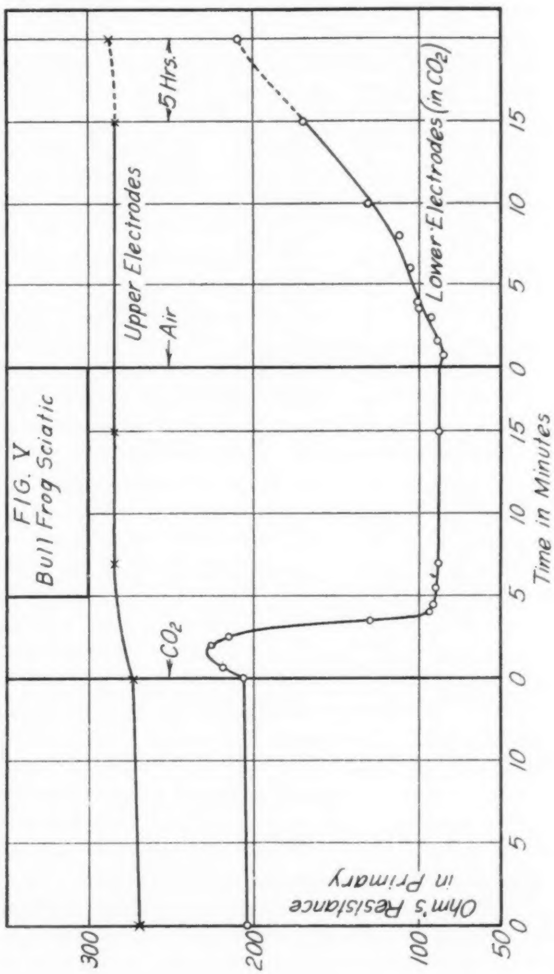
Green frog sciatic—gastrocnemius. April 23, 1929

CONDITION	LOWER ELECTRODES (CO_2 PORTION)				UPPER ELECTRODES			
			"Threshold"				"Threshold	
	Rheobase	Chronaxie	Resistance in primary circuit of coreless coil	Angle from vertical of secondary of cored coil	Rheobase	Chronaxie		
	volts	*sigma*	*ohms*	*degrees*	*volts*	*sigma*	*ohms*	*degrees*
Before CO_2	1.33	0.10	210	15	2.2	0.08	230	15
In CO_2, 16 minutes	2.05	0.10	130	20	2.2	0.10	220	14
After CO_2, 10 minutes	1.32	0.10	240	15	2.2	0.08	230	15

compartment. Stimuli from the fast coil applied to the exposed nerve, on the contrary, rapidly became ineffective. Responses at all leads fell to zero soon after CO_2 was admitted and returned when it was removed.

In most of the experiments, the response to a single stimulus was determined by the contraction of the attached gastrocnemius muscle, the nerve passing through a compartment which could be filled with CO_2 and was stimulated above or within this region. A typical experiment is shown in table 4. In nearly all cases, the threshold of the nerve above the exposed region was not modified; in the few in which it was increased, leakage of CO_2 into the upper section could not be excluded. Cooper (1924) found the region above CO_2 to be markedly affected. Changes in the least interval and the recovery curve (supernormal phase) at the "outside" electrodes were correlated with the presence of CO_2 below and were especially great after the "inside" electrodes had been used to stimu-

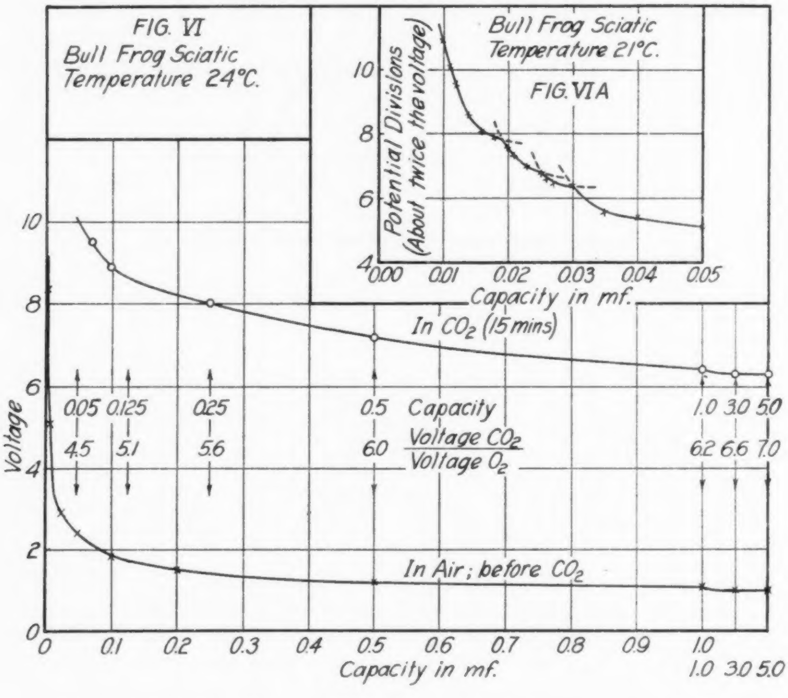
late. These findings are analogous to our results with action potentials—the leads proximal to the exposed region following the potential changes in the latter. It is not clear how far the changes she observed represent local



alteration at the outside electrodes nor how large a rôle actual diffusion of CO_2 may have played, but the appearance of a supernormal phase outside must be significant. We have not seen the outside threshold changes that

might be expected to accompany changes in the recovery curve, except as noted.

Within the CO_2 region, marked changes in irritability were rapidly manifest. The rheobase always rose, usually to more than double the normal value and in several instances over ten times, above the range of our voltage (10 volts at 10 m.F.). The chronaxie either did not change or more usually fell, even to one-fifth the original value. The threshold, as measured with a coreless coil, always rose, in the most extreme case to



three times the normal value. When carefully followed, there often appeared a transient fall in threshold in the first minute or two before the rise (fig. 5). The major changes were complete in two to four minutes after the admission of CO_2, though slight further drifts continued longer. In a number of experiments the maximum values were obtained in the first minute or two and later slowly fell during half an hour in CO_2.

On removing the CO_2 with an air current, the threshold voltages often showed a sharp further rise and then always fell toward the original values. This fall was much slower than the initial rise and often not complete for an hour or more.

The general effect of CO_2 is to raise the nerve threshold. It is apparent, however, that long enduring currents have become relatively less effective than short ones. Thus the rheobase, measured by the discharge of a 5 m.F. condenser has several times increased 5- to 10-fold when the voltage required by the coreless coil has only doubled or tripled. A fall in chronaxie with rise of rheobase means the same thing. To more exactly follow this change, the voltage-duration curve for threshold currents has been determined.[2] Figure 6 shows such curves for a nerve before and during exposure to CO_2 and illustrates the greater rise of threshold for the more enduring currents. Adrian (1920) observed the same effect with other acids. In occasional experiments (as when the chronaxie shows no change) the rise is symmetrical for all currents. Lapicque (1926) interprets a rheobase rise and chronaxie fall to lower membrane resistance; Lucas (1910) to rise in ion concentration required for excitation and change of ion mobility (colloidal change). We may well have all these effects to deal with in the case of CO_2 (see Boyd and Gerard, 1930).

In several experiments, though responses to stimulation at the upper electrodes remained vigorous throughout, responses to stimulation in the CO_2 stretch became very feeble. Small muscle responses at the new threshold level could not be increased by any amount of increase of stimulus strength. We do not now wish to insist on the theoretical consequences of such an observation, for it has occurred irregularly. Responses to stimulation at each set of electrodes have usually remained good, have sometimes failed at both, have suddenly fallen nearly to zero at both, or have fallen slowly or suddenly only at the lower electrodes while in CO_2 or more often while "recovering" from it. This failure of many fibres on returning from CO_2 to oxygen has been met not infrequently. The fact remains that we have observed, as a result of treatment with CO_2, that impulses initiated beyond it were conducted through a nerve stretch in which they could not be elicited by strongly supramaximal induction shocks. Considerably more work is needed to elucidate the conditions determining irritability and conductivity changes produced by CO_2; the conflicting experiences of those who have studied them attest the existence of unknown factors.

Discussion. We will not elaborate the discussion of the facts here reported. The influence of CO_2, possibly as a penetrating acid, on nerve potentials, refractory period, thresholds, etc., can be fairly well summarized in terms of *slowed* chemical reactions. These relations have been analysed in a preceding paper (Gerard, 1930) and need not be gone into again. If the views there developed be correct, slowing of a delayed recovery action

[2] When sufficiently close observations are taken, the simple curve shows a suggestion of breaking into a number of shorter ones (fig. 6A), as described by Kodera (1928) for European frogs.

must lead to the changes noted. There are, however, marked quantitative (if not qualitative) differences in the nerve response to asphyxia and equilibration on the one hand, and to CO_2 on the other, and other factors are probably operating in the latter case. It will be highly interesting to study the influence of CO_2 on the heat production of nerve and on its chemical state, especially the amount of phosphocreatine. The influence of CO_2 and HCO_3' on its oxygen consumption will be reported in another connection.

So far as studied, brain metabolism has appeared to be similar to that of nerve but very much more intense (see Gerard, 1928). The stimulating effect of CO_2, even in large concentrations, on many "centers" is well known and recently Loevenhart, Lorenz and Waters (1929) were able by CO_2 administration to arouse psychotics from long standing stupor, by means, they believe, of stimulation of cortical centers. Loevenhart has accumulated evidence that interference with oxidations in the cells of the respiratory center acts as a stimulus to them (Gasser and Loevenhart, 1913), and Gesell and his colleagues (1925) have shown that acidity in these cells provokes activity. There is considerable basis for generalizing to other nerve cells (see e.g., C. F. Schmidt, 1928), and probably to nerve fibers; and we should then expect CO_2 (acidity) to retard the oxidative reactions, as seems to be the case. Heinbecker (1929) has suggested specifically that acidity interferes with the oxidation of carbohydrates. This might be of importance in connection with the resting metabolism of nerve, but can hardly be the case for activity. Nerve does not burn sugar for its active metabolism (Holmes, Gerard and Solomon, 1930) and interference with resting reactions could hardly affect activity in the short time required for CO_2 to act.

SUMMARY

1. The influence of carbon dioxide on the action and resting potentials of isolated frog nerves has been studied with the aid of a moving coil galvanometer, as previously described. The rate of passing the gas through the nerve chamber was found to affect observations. Threshold changes to induction shocks and condenser discharges were studied on muscle nerve preparations or, using action potentials as the index of response, on isolated nerves.

2. Exposing a stretch of nerve to carbon dioxide causes a marked increase of total action potential, up to five times the initial values. This is true for carbon dioxide concentrations (in oxygen) from 10 to 100 per cent. The high responses are maintained during hours of exposure except in the case of 100 per cent CO_2 in which the responses fall to zero in two to three hours. This fall is probably due to asphyxia.

3. The rise is relatively much greater for stimuli at 90 a second than at 300, and is interpreted as due to prolongation of action potentials.

4. There is usually but little change in the responses below the exposed region so that few fibres can be blocked. The refractory period is increased.

5. On readmitting oxygen there is a further rise in action potentials to a peak value, 5 to 15 per cent above that reached during exposure to CO_2, followed by a fall to initial or somewhat lower values.

6. The first change in response on admitting carbon dioxide appears in 45 seconds, the maximum is reached in 20 minutes. These delays are probably not due to time required for diffusion into the nerve but measure the progress of a slow change initiated by carbon dioxide. On removing carbon dioxide, the first effect appears in 2 to 3 minutes, the peak is reached in 3 to 15 and an hour or more is required for the subsequent fall.

7. Similar but less marked changes are registered at electrodes leading from a stretch of nerve nearer the stimulating electrode, not exposed to carbon dioxide.

8. A nerve in carbon dioxide does not show the usual progressive increase of response (equilibration) with repetition of activity.

9. The resting potential is very sensitive to the rate of bubbling of gas through the chamber. When this factor is controlled, there still appears, when carbon dioxide is admitted, a sharp but brief rise in potential followed by a greater and maintained fall. On replacing carbon dioxide by oxygen there is a further brief fall and then a gradual rise to a new level, usually lower than at the start.

10. The threshold of the exposed region of the nerve, but no other, is markedly increased, sometimes after a preliminary fall, by carbon dioxide. It may be tripled for induction shocks and increased over ten times for long condenser discharges. The chronaxie is decreased and the voltage-duration curve altered, in that currents of shorter duration become relatively more effective than longer ones. The changes are reversed on removing the carbon dioxide. The rise after admitting the gas is complete in 2 to 4 minutes, the return to normal after its removal not for an hour or more.

11. These observations are interpreted as indicating a slowing of critical chemical reactions in nerve under the influence of carbon dioxide.

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DELAYED ACTION POTENTIALS IN NERVE

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The persistence of thermal and chemical changes in nerve for ten minutes or more after a short period of activity has been definitely demonstrated. The classical main action potential wave, lasting a few sigma, has been shown by many recent workers to represent only partially the potential changes of activity. Most striking, are the recent findings of Amberson and Downing (1929) that potential changes may persist twenty seconds or longer. These delayed changes have been interpreted by many (see Gerard, 1930) as representing recovery processes, and it might be anticipated that some electrical effect should also persist for minutes. Such late potential changes would manifest themselves as alterations of the resting potential and, indeed, such alterations have been described in the earlier literature (see Gotch, 1900). The experiments here reported demonstrate the existence of potential changes for as long as ten minutes after tetanization for one minute, though these have not been obtained on all nerves studied.

Methods. Green frog or bull frog sciatics have been used throughout, at room temperature (21°-23°). The distal end of the nerve was injured by crushing or boiling and the nerve placed with the injured end on one lead electrode, a second about half way along the side and the stimulating electrodes at the central end. The lead electrodes were freshly coated silver-silver chloride wires or the boot type with zinc rods dipping into zinc sulphate. A sensitive moving coil galvanometer was used as a null instrument, potentials across the electrodes being continuously balanced by means of a Pye potential divider. The circuit could be easily opened with a knife key. Slightly supramaximal make and break induction shocks at the rate of 300 a second were used for stimulation.

The balancing potential required at any moment is simply expressed in terms of the setting of the divider. As used, one division was approximately equal to one millivolt. Readings could be made to 0.01 division. Changes in the resting potential were followed for a period, no manipulation being carried out except moving the divider to maintain balance, then a period (30 to 60 seconds) of stimulation or polarization was initiated and changes in the resting potential again followed.

Results. A series of tests is shown in figure 1. It will be noted that following a period of tetanization, the resting potential shows a definite deviation from its base line of gradual change, and does not return to this line for some minutes. In one case shown (*A*, *1*) the deviation is in the direction of increased negativity, that is, following the main action potential there is a period of after-negativity. In the majority of experiments, and, indeed, in this same nerve later on, the after-effect is positive (*A*, *2*). The longest duration of after-potential with the nerve in oxygen is 12 minutes (*B 3* and *4*) at 22°C. The first reading possible, in some cases about five seconds after ceasing stimulation, shows the maximal deviation and the after-potential then falls, rapidly at first, until the resting level is again attained.

With the nerve in carbon dioxide, the after-potential is greatly modified. It is still in the positive direction but is more delayed so that the rise as well as the fall are prolonged and the former can be followed (*B 5*, *6*, *7*). The first reading after a period of activity may show little change, the after-positivity then rising over some minutes and finally again disappearing.

It seems to be established that the action potential is markedly prolonged in carbon dioxide (see Amberson and Downing, 1929; Necheles and Gerard,

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Fig. 1. *A*. Green frog sciatic. November 15. 23°C. Ag = AgCl electrodes. *1*, 30 seconds tetanus (deflection = −43); 5 hours after dissection. No gas bubbling. *2*, 30 seconds tetanus (deflection = −40) 2 hours after *1*. One division in balance potential is equal to about 1 mv. and an unbalance of 1 division gives a galvanometer deflection of about 10 mm. with this nerve.

B. Green frog sciatic. November 16. 22°C. Ag. = AgCl electrodes. Twenty-four hours (at room t°) after dissection. End not crushed. *1*, 60 seconds tetanus (deflection = −18). *2*, 60 seconds tetanus—circuit open during tetanus. End of nerve killed by boiling water. Next test in half an hour. *3*, 60 seconds tetanus (deflection = −100). *4*, 60 seconds tetanus—circuit upen during tetanus. CO_2 in all parts of chamber. Next test in 10 minutes. *5*, 60 seconds tetanus (deflection = −110). *6*, 60 seconds tetanus (deflection = −140). *7*, 60 seconds tetanus (deflection = −75; at end of tetanus, deflection = +60). Three hours after *6*. *8*, 60 seconds polarization (deflection = −85, by raising balancing potential 6.5 divisions). Chamber opened and CO_2 blown out. Large increase of resting potential and then fall. First test 25 minutes after opening, fall still rapid. *9*, 60 seconds tetanus (deflection = −60; at end of tetanus, deflection = +155).

C. Green frog sciatic. November 18. 23°C. Zn = $ZnSO_4$ electrodes. Six hours after dissection, first test 15 minutes after end crushed. *1*, 60 seconds tetanus (deflection = −50). *2*, 60 seconds tetanus (deflection = − 200). Two hours later. *3*, 60 seconds polarization (deflection = −200, by raising balancing potential 7 divisions). String soaked in Ringer in place of nerve. *4*, 60 seconds polarization (deflection = −200, by raising balancing potential 3 divisions).

D. Bull frog sciatic. November 19. 21°C. Ag = AgCl electrodes. Three hours after dissection. First test 40 minutes after end crushed. *1*, 60 seconds tetanus (deflection = −155). *2*, 60 seconds tetanus, no deflection, action potential balanced by lowering balancing potential 2.5 divisions.

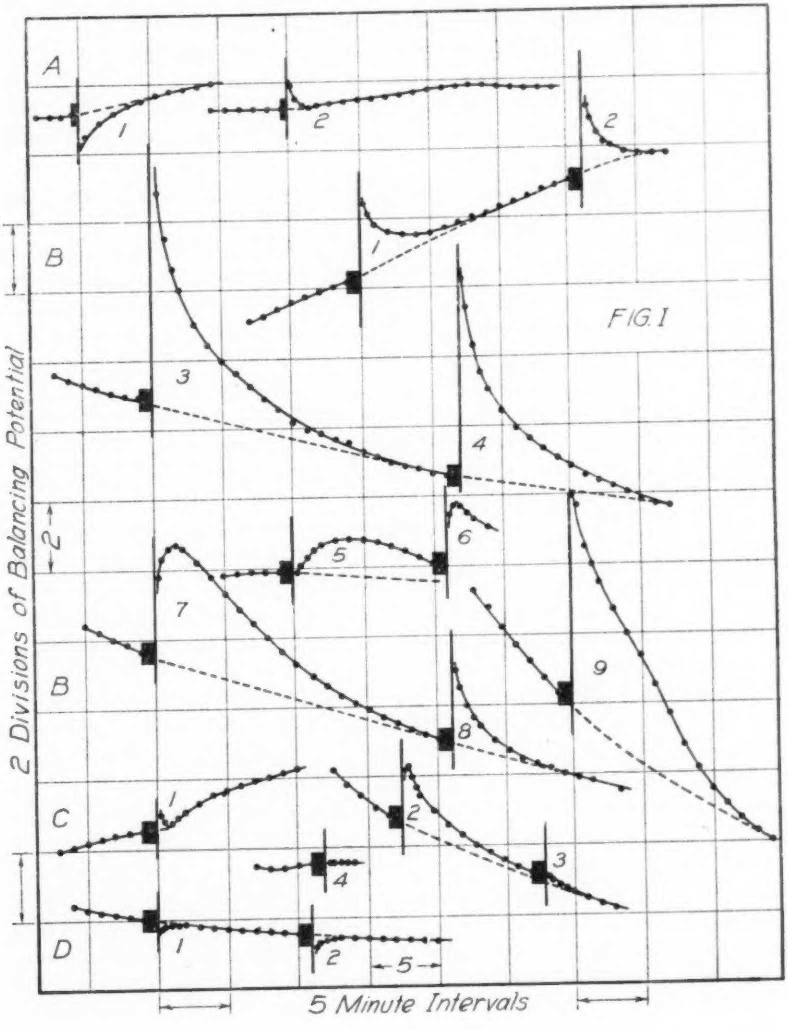
A
1
2
2
B
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FIG. I
3
4
2
6
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7
B
9
8
C
1
2
4
3
D
1
2
5
2 Divisions of Balancing Potential
5 Minute Intervals

Fig. 1

1930) so that there is a negative after-potential for some seconds. In air, an after-positivity appears in a shorter time. The present findings indicate that in carbon dioxide also an after-positivity appears but so delayed that its rise can be followed over minutes.

The magnitude of these after potentials, as can be seen from the figure, is by no means inconsiderable. Maximum readings have been as great as one-third of the potential change during tetanization. The after-potentials seem to be more pronounced in nerves that have stood some hours than in fresh ones (*C1* and *2*).

It is obvious that, in such experiments, there is great danger of error due to simple polarization. It is almost impossible to obtain strictly non-polarizable silver-silver chloride electrodes, and even the zinc sulphate boots are often far from perfect. To some extent the after-effects here described are undoubtedly due to electrode polarization. There are several reasons for believing, however, that a genuine biological effect is also present and that the polarization potentials may be corrected or eliminated. 1. Following a potential imbalance and current flow, due to the side of the nerve becoming less positive, the polarization potential should cause a reversal—giving an after-positivity. In several observations the after-potential was negative and the same nerve, with no alteration of contracts or circuit, has given first negative and later positive after-potentials. 2. Electrode polarization can be eliminated by keeping the outside circuit open during the period of tetanization and potential unbalance, so that no current flows between the nerve and electrodes. Simply opening the circuit for sixty seconds with the nerve at rest does not lead to any potential change; but when the nerve is tetanized during this minute the same after-potential changes are observed as when the circuit is left closed (*B 1*, *2* and *3*, *4*). 3. Instead of preventing current flow through the electrodes during tetanization by opening the circuit, this may be achieved by proper change in the balancing potential. The usual current flow through the outer circuit during activity, from end to side, may be over-compensated by change in the balancing potential so that a small current flows in the opposite direction. This should abolish or reverse after-potentials due to electrode polarization but it does not abolish those observed after nerve activity (*D 1* and *2*). 4. The potential difference between the electrodes normally appearing during activity of the nerve, and the consequent current flow, can be produced by altering the balancing potential. The electrodes and the resting nerve can thus be "polarized" to the same extent as during activity. Such tests show that some polarization may occur, even when a Ringer-soaked string is used in place of the nerve. The after-potential change is usually much less after such a polarization than after a tetanization giving an equal deflection for an equal time; and the after-potential has been almost absent after polarization at a time when a definite one was

present following activity (*B 7, 8, 9* and *C 2, 3*). 5. It is difficult to see how the presence of carbon dioxide could greatly alter the form of the after-potential if it were due to electrode polarization, especially so as to cause the after-potential to increase for some time after the activity is ended. 6. It may be added that the after-potentials cannot be attributed to the stimulating currents, via electrotonic currents or by other means, since these effects do not follow greatly increased stimuli when nerve conduction has been in any way interfered with. When there are no action potentials there are no after-potentials.

The above considerations take account of electrode polarization but do not all apply to polarization at axone membranes or the nerve sheath. The existence of after-potentials in the same direction as the main action potential change, and the gradually increasing after-potentials seen in carbon dioxide, however, do speak against simple polarization effects as being their source. Sheath polarization lasting ten minutes or longer is very improbable, especially since an outside polarizing current must also affect the sheath and the after-potentials of such currents are of much shorter duration. As regards the axone membrane, it may be added that polarization seems to be one of its major attributes and any changes in such polarization of functional significance. The existence of such prolonged potential changes lends further support to the view that, after the initial explosive reaction of conduction, there is a prolonged recovery period in which the nerve membrane participates. The relations of thermal and metabolic changes in nerve to the electrical ones have been previously discussed from this viewpoint (Gerard, 1930).

SUMMARY

The main action-potential accompanying nerve activity is followed by after-potentials (deviation from the resting potential) that may last over ten minutes. They are usually, but not always, an after-positivity.

Carbon dioxide delays the appearance of and prolongs the after-positivity.

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STUDIES ON NERVE METABOLISM

VI. The Carbohydrate Metabolism of Active Nerve

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In a previous paper of this series (Holmes and Gerard, 1929) it was shown that the "free sugar," but not the glycogen, present in the isolated rabbit's sciatic nerve decreased during rest in oxygen. There was no change in the lactic acid content. Assuming that the carbohydrate lost was oxidized, it would account for 60 per cent or more of the oxygen consumption.

Gerard (1927), Fenn (1929), and Meyerhof and Schmitt (1929) have alike found that the R.Q. of frog nerve is increased during stimulation, the R.Q. of the extra metabolism being 1.0 or more. Though it was pointed out (Gerard, 1927) that the oxidation of carbohydrates as the energy source of activity is not a necessary consequence of the R.Q. findings, analogy with the known changes in muscle and other tissues made this seem the most likely inference. We were, therefore, much surprised to find that the carbohydrate changes of rest were unaltered by activity. We state this result with considerable assurance since it was established independently in the two laboratories on different nerves and by different methods.

Methods. a. *Nerves.* Rabbit sciatics were dissected as previously described (Holmes and Gerard, 1929). Bull frog (R. catesbiana) sciatic, and usually brachial, nerves were obtained in a similar manner. Nerves already dissected were kept in a dry mortar on ice while subsequent ones were removed, the completed batch was then hung in moist oxygen and left at rest or stimulated for a determined time and finally returned to the same iced mortar for grinding.

b. *Tests for activity.* It was important to make certain that the nerves remained in good condition and able to conduct impulses during the period of stimulation, since absence of change on stimulation might result from the nerve's inability to respond. Bull frog nerves, in our hands, have remained fully active for 24 hours or longer as tested by their action potentials

(Gerard, 1930). In several of the present experiments the nerves were tested at the end of the run and good sized potentials obtained. The rabbit nerves caused some concern, since mammalian nerves are more susceptible to injury than those of the frog and might also be seriously affected by their sojourn on ice. We have found their resting oxygen consumption to be entirely uninfluenced by a preliminary cooling, and action potentials, determined as above, were well maintained over several hours. One of us (E. H.) erected an amplifier acting into a voltmeter and followed the action potentials throughout the period of stimulation, with the same results. The details of this arrangement are given in an appendix.

The rabbit experiments were carried out in a thermostat at 37°C. The frog nerves were run at 19°–23°C., the temperature remaining constant for each experiment.

c. *Stimulation.* In all experiments, definitely but not greatly supramaximal induction shocks were used to stimulate. The primary circuit was interrupted either in the usual manner by a buzzer on the coil giving 100 shocks (make plus break), 200 shocks or 280 shocks per second, or by a rotary interrupter giving up to 1000 shocks per second. In several experiments the induction coil was activated by a 90 cycle A.C. current.

A frog nerve stimulated continuously with 280 shocks per second uses only one-third as much extra oxygen per minute of stimulation as when stimulated for short intervals with ample rests between (Gerard, 1927). The maximum extra usage per unit time is, of course, attained with continuous stimulation. Since there are no similar data for rabbit nerve yet available, we have used several sequences of stimulation in these experiments, varying from continued tetanization to about two minutes' stimulation to one of rest.

d. *Chemical analysis.* One systematic procedure has been fully described in the previous paper (Holmes and Gerard, 1929). Where present results are given in terms of total carbohydrate rather than glycogen and free sugar, the earlier form of the method was used.

A new procedure has also been used and found very satisfactory. The iced nerves are ground and extracted five times with 1 cc. portions of ice water. To the combined extracts alcohol is added to a concentration of 80 per cent and the whole kept at room temperature over night. The precipitate is centrifuged down and washed three times with 2 cc. and once with 5 cc. portions of alcohol. The residue (plus the original nerve débris as desired) is analyzed for glycogen as previously, the fluid for lactic acid and free sugar.

The fluid is carefully evaporated on a sand bath, with several additions of water, to a volume of 1 cc. (The final concentration is carried out in a graduated centrifuge tube.) One cubic centimeter of the $HgSO_4$-H_2SO_4 reagent of West, Scharles and Peterson (1929) is added and the whole al-

lowed to stand over night. The precipitate is centrifuged out, washed twice with $\frac{1}{2}$ cc. portions of the reagent and twice with 1 cc. of water. The combined fluids are treated with solid $BaCO_3$ until the precipitate is faintly yellow, filtered and well washed and the mercury removed in the usual manner with H_2S followed by aeration. The final solution is made up to 15 cc., of which 10 cc. are used for the lactic acid determinations, the remainder for free sugar. Lactic acid is determined by the method of Friedemann, Cotonio and Shaffer (1927), sugar by a modification of the Shaffer-Hartman copper method.

TABLE 1

NERVE A	FOUND		NERVE B	ADDED		FOUND		RECOVERED	
	Lactic acid	Free sugar		Lactic acid	Glucose	Lactic acid	Free sugar	Lactic acid	Glucose
mgm.	*mgm. per cent*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
345	70	118	335		0.5	65	266		99.0
			—		0.5				99.5
458	70	100	388		0.5	72	227		94.5
267	75	105	259	0.4	0.2	211	181.5	88.5	99.2
390	70	98	455	0.3	0.2	128	143	88.3	102

TABLE 1A

RABBIT NERVE	"OLD" METHOD—ALCOHOL EXTRACTION			"NEW" METHOD—WATER EXTRACTION		
	Free sugar	Glycogen	Total	Free sugar	Glycogen	Total
Initial	48	53	101	85	25	110
2 hours' rest in O_2, 37°C	22	56	78	66	25	91
Difference	−26	+3	−23	−19	0	19

This procedure is considerably more rapid than the other and gives somewhat more consistent figures. It also permits the determination of all three substances in each sample. We are not certain that the separation of glycogen and free sugar is quite so satisfactory, but in the present experiments the exact distribution of carbohydrate between these two fractions is of little consequence.

We have been able by this procedure to recover 99 per cent of glucose from pure solution or when added to nerves. Lactic acid is lost during the separation, only 88 per cent being recovered, but this loss is consistent enough to permit the use of a correction factor. (The results as tabulated throughout have not been corrected.) Table 1 indicates the type of control results obtained. All bull frog experiments were done by this

method, rabbit experiments by the earlier one. Rabbit nerves analyzed by the later method gave higher values for free sugar and lower ones for glycogen than by the earlier one; the totals, however, remained alike (table 1a).

TABLE 2

A. Values in milligrams per cent

NERVE	AT ONCE	REST IN O_2 (HOURS)						
		1 hour	2 hours	3 hours	5 hours	7 hours	9 hours	Average rest
	Lactic acid							
Bull frog* (19–23°C.)	72 (10)†			69 (9)	76 (2)	73 (6)	76 (3)	72 (30)
Rabbit (37°C.)	‡81 (26)	82 (4)	83 (14)					
	Free sugar							
Bull frog (19–23°C.)	91 (8)			74 (10)	61 (2)	47 (4)		
	Total sugar							
Rabbit (37°C.)	‡101 (26)	68 (1)	50 (2)	43 (1)				

* Frog analyses by new method, rabbit analyses by earlier one.

† The number in brackets gives the number of experiments averaged.

‡ These values taken partly from the previous paper.

B. Values in milligrams per cent

REST IN O_2	DECREASE IN CARBOHYDRATE			
	Bull frog		Rabbit	
	Total	Per hour	Total	Per hour
hours				
1			33	33
2			51	25
3	16	6−	58	19
5	30	6		
7	44	6+		
8.5	57*	6		

* Taken from six earlier experiments, during May, not included in the above table, which was made from experiments carried out during the summer months. The initial free sugar in these averaged 120 mgm. per cent but the values were very scattered.

RESULTS. a. *Rest.* The changes in the carbohydrate elements of the bull frog and rabbit nerve during rest are summarized in table 2. It is interesting to compare them with those of rabbit nerve, previously re-

ported. Qualitatively the two are entirely similar. In both cases, the lactic acid content is unaffected by rest in oxygen for several hours and, on the basis of fewer experiments, the same appears true for the glycogen. The free sugar, in contrast, shows a definite fall.

The rate of disappearance of free sugar is practically constant for the bull frog nerve, at least up to nine hours, 6 mgm. per 100 grams being lost per hour at 22°C. Our previous experiments with rabbit nerve did not indicate carbohydrate loss beyond the second hour, but the present additional data show that for this tissue also, sugar is still disappearing more than three hours after dissection. The rate appears to decrease with time, as in fact it must. At the initial rate of disappearance, all free sugar would be gone from rabbit nerve in about two hours (at 37°), and even with the falling off observed, it is about gone in three. It is interesting to note in this connection that the resting metabolism of these nerves, as measured by their oxygen consumption, is maintained at a constant level for much longer periods than these. During the first hour, the sugar disappearing, if oxidized, could account for nearly all of the oxygen consumed; later, it could account for almost none. The tissue, then, is able, if it burns sugar at all, to substitute another fuel with no break or disturbance in its chemical dynamics.

An even more striking situation is encountered in the bull frog nerve. The average oxygen consumption of these nerves in over thirty experiments (R. W. G.) is 30 cmm. per gram and hour at 22°C., and this rate is maintained for more than twenty-four hours. If all used for oxidizing sugar, this would cause the loss of 4.0 mgm. per cent per hour, or 100 mgm. per cent in 25 hours. This is more than the entire "free sugar" content of nerve, part of which is not free hexose. The actual rate of disappearance of "free sugar" under the same conditions is 6 mgm. per cent per hour or 50 per cent more per unit time than could be oxidized by all the oxygen taken up in the same time. The rabbit sciatic at 37° uses oxygen nine times as rapidly as the bull frog's at 22° but uses free sugar only three to six times as rapidly.

b. *Stimulation.* The results on bull frog and rabbit nerves (tables 3 and 4) are entirely consistent in showing the absence of effect of stimulation. Glycogen values remain steady in oxygen during rest or activity, and free sugar values fall to the same extent in both cases. The possibility suggests itself that, for the longer periods, the sugar is so completely used up even at rest that no extra usage due to activity could be observed. A number of experiments over shorter time intervals, however, have shown the same results, although further loss of sugar was clearly possible. The lactic acid content of stimulated nerves compared to resting showed a slight fall in the rabbit series and a somewhat greater rise in the frog series. Statistically, the rise appears to be significant, but it is not accompanied

by a corresponding fall in carbohydrates. It is due largely to one series of experiments, however, in which lactic acid rose and sugar fell in the stimulated nerves. The nerves here were especially large and packed together for stimulation and it is possible that oxygen diffusion to the inner ones was not adequate for the requirements during activity, with a resulting partial asphyxia and lactic acid formation.

TABLE 3

Bull frog. Values in milligrams per cent

DATE	TEMPERATURE	DURATION	LACTIC ACID		FREE SUGAR		GLYCOGEN	
			At rest*	Stimulated*	At rest	Stimulated	At rest	Stimulated
	°C.	hours						
May 16	23	6			(112)	(123)	28†	28
May 20	22	3	80	82	58.5	61		
	22	5	74	77	51	55		
May 25	20	7	72	75	65	68	24‡	25
May 26	19	8	80	71	64	51		
	19	8	79	73	69	78		
	19	9	80	85.5	69	66		
	19	9	73.5	69	70.5	77		
June 3	21	3	75	63.5	94	90	20	19.5
July 22	22	3	47.5	60	74	61		
	22	3	70	88	92	61		
July 23	22	3	60	80	79	70		
July 24	22	4	79	97	58	66		
July 25	22	3	75	78	74	77		
	22	3	70	75	77	80		
August 23	22	3			72	69		
	22	5			64	62		
	22	7			49	48		
September 4	22	7	89	97	57	52		
September 8	22	3	61	85	71	70		
	22	5	79	82	58	57		
Average			72.5	77.4	70.7	69.7		

* All "rest" and "stimulated" values are on pairs of nerves from the same frogs. Continuous stimulation, 100 shocks per second.

† Another pair of glycogen values is: at once, 28; at rest 6 hours, 28.

‡ Another pair of glycogen values is: at once, 25; at rest 8 hours, 25.

DISCUSSION. a. *Rest.* The results with bull frog nerve show that some of the sugar which disappears during rest is not immediately oxidized, since, in the early periods, more carbohydrate disappears than could be oxidized by the oxygen consumed during the same time. A sufficient time after isolation of rabbit or frog nerve, however, though the oxygen uptake continues, carbohydrate disappearance has ceased. In neither case is there direct evidence of sugar oxidation.

It is not certain that the "free sugar" lost represents glucose, though we have excluded certain non-carbohydrate reducing substances, such as creatinine. In favor of it being glucose is the fact that much of it changes into lactic acid under anaerobic conditions. Assuming, for purposes of argument, that the substance really is glucose, loss of reducing power would result from its conversion into hexose phosphate. We may recall that Gerard and Wallen (1929) found, in frog nerve, after long rest in oxygen, an average increase of 1 mgm. of acid-stable phosphate per 100 grams of nerve. This could account, at the most, for 6 mgm. of hexose converted

TABLE 4

Rabbit. Values in milligrams per cent. All experiments at 37°C.

NUMBER	DURATION		LACTIC ACID		FREE SUGAR		GLYCOGEN		TOTAL CARBOHYDRATE	
	Total	Stimulation	At rest	Stimulated	At rest	Stimulated	At rest	Stimulated	At rest	Stimulated
	minutes	*minutes*								
1*	60	60	102	108					68	66
2	160	90	64	47					43	37
3	109	77	78	82					47	41
4	124	67	152	152					53	44
5	130	86	66	55					—	(50)
6	350	143	61	56					38	36
7*	102	81	68	64			56	55		
8*	135	98	53	53			51	59		
9*	140	61	77	81			36	34		
10*	135	109	73	70	23	19				
11*	120	90	—	—	21	18				
12*	100	90	93	94	11	16				
Average Nos. 1–6			88	83					50	45
Average Nos. 7–12			73	72	18	18	48	49	66	67
Average All			80	78					58	56

* Continuous stimulation. In others alternate periods of stimulation and rest, of varying lengths.

into hexose monophosphate, which would give a loss of reducing power of 2 mgm.—far too little to account for the observed excess loss of "free sugar". Conversion of sugar to fat is precluded by the resting R.Q., which several observers find to be less than 1.0.

In the present state of our knowledge, the most plausible explanation seems to be that, especially during the first few hours, some glucose is converted into a substance with less, or no, reducing power, by a change that requires little or no oxygen. Thus, for example, Shaffer and Friedemann (1924) have described the reaction of glucose and aceto-acetic acid to give a non-reducing condensation product. Or, again, a little oxygen

might cause a large loss of reducing power by oxidizing glucose to an intermediate, such as gluconic acid. It is possible that such a substance later undergoes further oxidation. The difficulty remains, in any case, that the accumulation of an intermediate product or its formation at one time and oxidation at another indicates the absence of a resting equilibrium in isolated nerve. This might be an expression of interference with the normal chemical exchange between nerve and blood, of the inevitable injury of removal, or of some irreversible degeneration process due to separation from the cell body.

b. *Activity.* Whatever carbohydrate is oxidized or otherwise altered at rest, our results give no evidence that the situation is changed during activity. It is conceivable that activity causes a greater fraction of the free sugar disappearing during rest to be oxidized, but it is a very unlikely assumption; and since activity is still possible long after the "free sugar" has practically disappeared, such an assumption has no virtue. We conclude, then, that the nerve does not obtain its energy for activity by the oxidation of free sugar, glycogen or lactic acid. Previous work (Gerard, 1927) has shown that this energy does come from oxidations, so the usual carbohydrates seem to be out of the picture. The possible oxidation of the carbohydrate elements of the cerebrosides is being investigated by one of us (E. H.). The breakdown of phospholipins on activity was suggested by the findings of Gerard and Wallen (1929). An extra ammonia liberation during activity has been reported by several workers (Tashiro, 1922, Winterstein and Hirschberg, 1925, Gerard and Meyerhof, 1927), and the latter have calculated that if this ammonia resulted from protein oxidation, the protein oxidized agrees well with the extra oxygen consumed. It seems more probable, however, that the ammonia arises by deaminization of a nucleotide, as found in the case of brain (Pohle, 1928). The question is being further investigated for nerve. The R.Q. of activity is higher than that of rest by about 0.2, though there is uncertainty as to the exact value. This is the only clue we now have as to the fuel used by nerve for doing its work.

SUMMARY

1. The "free sugar," glycogen and lactic acid content of rabbit and bull frog nerves have been determined by two methods.

2. During rest in oxygen there is no change in glycogen or lactic acid content. The free sugar decreases with increased duration. In rabbit nerves at 37°C. the decrease is 36 mgm. per cent for the first hour, progressively less during later periods. In bull frog nerves at 22°C. the rate of fall is constant for at least nine hours at 6 mgm. per cent per hour. This is a loss of 50 per cent more "free sugar" than could be oxidized. Its fate is unknown.

3. Nerves stimulated in various ways and for periods up to nine hours have the same content of free sugar, glycogen and lactic acid as their resting controls. It is concluded that the extra energy required for nerve activity is not obtained by the oxidation of any of these substances.

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Appendix. The method for observing the electric responses of the rabbit nerves gave rise to some difficulty, since a galvanometer, sufficiently sensitive to record the "negative" variation was not available. In view of the high price of such an instrument, it was decided to employ a valve amplifier, since the necessary "wireless" components are comparatively cheap, and easily obtainable. A three valve amplifier, similar in general plan to that described by Adrian[1] and Matthews[2] was constructed, and I would thank Doctor Adrian and Doctor Matthews for help and advice in the matter. To Mr. C. R. Cosens of the Engineering Laboratory, Cambridge, I am indebted for continued practical help and advice, as well as for the loan of apparatus.

The details of the instrument in its final form were as follows. Three "Cosmos" A. C. "Green Spot" valves were arranged with condenser—capacity coupling. 0.01 m.f. condensers and 0.25 megohm grid leaks being used. The anode resistances were of wire-wound type, of value 60,000 ohms; those to the first two valves were arranged on the Feranti anode feed scheme; a 10,000 ohm resistance was connected in series with one of 50,000 ohms, and the point of junction between the two was earthed through a 2 m.f. condenser.

The anode of the last valve was connected through the "output" plug, and a ½ m.f. condenser, to the outer grid of a 4 electrode valve arranged as cumulative grid rectifier. A milliameter in the anode circuit of this gave a kick, when the electric response of a live nerve was applied to the input of the apparatus, which was several times larger than that caused by leak of stimulating current down a dead nerve. The amplifier was completely enclosed in a zinc box, which was connected to earth.

The following arrangement was adopted to ensure that the nerves were kept moist, and at 37°, during the experiment, to apply the stimuli, and to lead off the electric response.

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[1] Adrian: 1926. Journ. Physiol., lxi, 65.
[2] Matthews: 1928. Journ. Physiol., lxv, 222.

An ebonite pillar was mounted on a base of the same material. The pillar carried two ebonite cross pieces mounted on collars, which could be clamped in any desired position on the pillar by ebonite screws. The upper crosspiece carried the stimulating electrodes—two pipe cleaners, one end of each of which was soldered to a socket into which the stimulating leads could be plugged. The woolly parts of the pipe cleaners were saturated with Ringer's solution. The lower cross piece carried a third pipe-cleaner, which was connected to earth through a piece of thin enameled copper wire. Four pairs of "lead off" electrodes were mounted on the base; each was "U" shaped, and of the silver-silver chloride-Ringer-wool type; they were clamped in place by an ebonite strip.

The apparatus, thus far described, was mounted on a sheet of plate glass. Four holes drilled in the glass carried rubber bungs, sealed in with paraffin wax, through each of which passed a pair of the silver wires from the "lead off" electrodes; another one, on the opposite side, gave entrance to the stimulating electrodes, and to the earth lead.

On one side of the wooden base were sockets into which could be plugged the stimulating leads. On the opposite side, eight sockets, mounted on ebonite, allowed connection to be made between the silver wires from the "lead off" electrodes and eight plugs, the leads from which terminated in other sockets conveniently placed for connection to the input lead of the amplifier. These plugs were mounted on a single piece of ebonite, so that they could be connected or disconnected by a single movement.

A glass bell jar, the rim of which rested on the glass plate, covered the apparatus, and could be sealed with vaseline—rubber tap grease, so that it made an air-tight chamber. The hole in the top was closed by a rubber bung, which carried 1, a glass tube with stopcock for admitting gas; 2, a glass exit tube, which could be closed with rubber tubing and screw clip; 3, a pair of leads to a 100 volt 24 watt electric bulb fixed permanently against the inside of the jar, which was used for preliminary adjustment of temperature; 4, a third glass tube, which enclosed a constantan-copper thermocouple.

The whole apparatus was placed in an incubator, kept at 37°. Two separate holes in the top gave entrance, one to the leads to the stimulating electrodes, warming lamp, and thermo-couple; the other to those to the lead off electrodes. All leads were sheathed in metal, and the sheaths were earthed, as was the copper jacket of the incubator. The sockets connected with the lead off electrodes were screwed permanently on to the outside of the incubator. A piece of "armoured flex" ending in a plug permitted any one of them to be connected to the amplifier. The rather elaborate shielding of apparatus and leads was rendered necessary by the proximity of the A. C. mains.

The nerves were dissected out with the precautions previously described, and one group was placed on the electrodes, that leading to earth making contact with the middle of the nerve; along with them was placed a dead nerve. The other, or unstimulated, group was hung from the upper ebonite crosspiece, clear of the electrodes. Cotton wool, soaked in warm water, was packed round the ebonite frame work, and a sheet of wet cotton wool was arranged to shield the nerves from the direct rays of the lamp. The apparatus was placed in the incubator, and filled with oxygen, which was bubbled through warm water. The incubator was closed, the lamp switched on, and the temperature of the apparatus brought to 37°. This usually took 10 to 15 minutes. The temperature was read off from the thermo-couple, the cold junction of which was kept in a "thermos" full of ice. The galvanometer (Pye, moving mirror type) was adjusted, with a series resistance, to read 1 cm. on the scale per degree centigrade.

An ordinary "student's" induction coil was used for stimulation. The current for the primary was led off from a potentiometer, and single break shocks were obtained by closing a key which short-circuited the terminals of the primary, thus obviating sparking. The stimuli were adjusted until a full scale deflection of the milliameter was obtained from the live nerves, while the dead nerves were showed only a feeble kick, or none at all.

THE CAPILLARY BLOOD PRESSURE IN MAMMALIAN MESENTERY AS DETERMINED BY THE MICRO-INJECTION METHOD

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Micro-injection studies of the circulation in the frog's mesentery (Landis, 1926) indicated a balance between capillary blood pressure and the osmotic pressure of the plasma proteins. Direct quantitative measurements of the fluid interchange in single capillaries (Landis, 1927) showed that the rate at which fluid passes through the capillary wall is directly proportional to the difference between capillary pressure and the colloid osmotic pressure of the blood. The fall in blood pressure through the peripheral vessels of the frog is such that average blood pressure in the arteriolar portion of the capillary network exceeds the osmotic pressure of the plasma proteins, producing in this area filtration of fluid into the tissue spaces. In the venous capillaries, however, absorption takes place since the average blood pressure in this region is below the osmotic pressure of the plasma proteins. These experiments provide direct evidence in favor of the Starling hypothesis of fluid interchange through the capillary wall of the frog.

In mammals, however, the osmotic pressure of the plasma proteins is over twice as great as that observed in the frog (Starling, 1896; Moore and Roaf, 1907; Krogh, 1929, and others). If fluid interchange in mammals is governed by the same physical factors, capillary blood pressure must be correspondingly high in order to maintain the balance between filtration and absorption. This paper records direct measurements of arteriolar, capillary, and venous capillary blood pressures in the mesenteries of the rat and guinea pig, with determinations of the osmotic pressure of the plasma proteins in the same animals.

Material and methods. Decerebrate rats and anesthetized guinea pigs were used. Rats weighing between 150 and 230 grams were lightly etherized, the carotid arteries ligated and the trachea cannulated. The top of the cranium was removed and the cerebral hemispheres carefully scooped out. In several instances the blood in the superior longitudinal

sinus was coagulated by means of a cautery. The loss of blood was usually slight, but if significant hemorrhage occurred the animals were not used for determinations of blood pressure gradients.

For the guinea pig a general anesthetic was necessary because violent leg movements continued after decerebration. Veronal in minimal dosage was used, reënforced by ether while the abdominal wall was incised. The veronal anesthesia alone was sufficient as long as the mesentery was not stretched.

With the animal lying on its back, a loop of small intestine was withdrawn through an incision in the lateral portion of the abdominal wall and the mesentery was laid loosely over a transparent glass stage in a manner similar to that described earlier for the frog. The exposed mesentery was covered by a continuous flow of mammalian Ringer's solution, saturated for the majority of the experiments with a mixture of 20 per cent carbon dioxide and 80 per cent oxygen. Later it was observed that the addition of carbon dioxide to the fluid was not essential, so that simple oxygenated Ringer's solution was used. The oxygenation of the irrigating fluid seemed to be of the highest importance, since capillary stasis resulted in twenty to thirty minutes if the tissue was covered with imperfectly aerated fluid. In this respect the mammalian capillaries in the exposed mesentery seemed to be even more sensitive to lack of oxygen than the capillaries of the frog (Landis, 1928).

The Ringer's solution was delivered to the mesentery at a temperature between 36 and 38°C. by means of a fine glass tube placed close to the tissue. In addition a thermocouple of the type devised by Bazett and McGlone (1927) was laid on the mesentery in order to check the temperature at intervals.

The blood pressure in single arterioles, capillaries and venous capillaries was measured by the micro-injection technique described in detail in an earlier paper (Landis, 1926) using the Chambers micro-injection apparatus combined with a device for measuring the pressure of injection in centimeters of water. The small diameter of the capillary vessels in the mammal required the use of extremely fine pipettes, most of them less than 5 micra in total diameter at the tip. The micro-pipette was inserted in each instance into a side branch of the capillary, the pressure changes of which were to be measured. The pressure in such an occluded collateral was equal to the lateral pressure in the straight vessel. Capillary pressure could thus be measured repeatedly while blood flow in the observed capillary proceeded uninterruptedly throughout the entire period of observation. After the pipette was introduced a few corpuscles were drawn out of the main stream back into the vessel functioning as a side tube. Intermittent movement toward or away from the pipette occurred synchronously with the heart beat when the pressure in the water column of the

apparatus was below or above mean capillary pressure respectively. At equilibrium, when the pressure of the water column exactly balanced capillary pressure, the corpuscles oscillated back and forth about the same point, and capillary pressure could then be read directly from the column of water.

In this way blood pressure was measured in a series of capillaries, as near their origin from the arteriole as possible. This was followed by determinations in the venous capillaries and in the arterioles of the same area. The tip of the micro-pipette was then broken off to provide a larger tube (40 to 50 micra) for introduction into the artery and vein at the base of the mesentery. In these it was necessary to determine the pressure which would barely prevent the advance of blood into the micro-pipette. The rapid pulse rate, usually above 150 per minute, made it impossible to determine more than mean pressure. Aortic pressure was measured at the end of the experiment in the usual manner with a cannula inserted into one of the iliac arteries or into the common carotid.

For the determination of the osmotic pressure of the plasma proteins the blood was withdrawn by cannula from the aorta as rapidly as possible into a syringe containing 2 drops of 5 per cent potassium oxalate solution. The corpuscles were thrown down by centrifuge and the plasma placed in a small collodion sac of about 2.0 cc. volume, which was then attached, after the method of Mayrs (1926), to a glass tube of fine bore which was filled with plasma to a height of 25 cm. The collodion sac,[1] filled with plasma, was immersed in a large volume of mammalian Ringer's solution and left to come into equilibrium at room temperature. Equilibrium was complete in 12 hours, and was tested by lowering the level of the plasma in the tube 1 cm. and observing the slow rise of the meniscus with a microscope. The addition of plasma to 1 cm. above the point of initial equilibrium produced a downward movement of the meniscus. An approximately equal rate of movement in these two instances indicated that equilibrium had been obtained with sufficient accuracy for a comparison with the capillary blood pressures observed. In most instances the height of the column of plasma was again read after 18 hours, i.e., six hours after the first observation, but there was no significant change in any instance.

OBSERVATIONS. *a. The blood vessels of the mammalian mesentery.* The mesentery of the rat and guinea pig is not as richly vascular as that of the frog. The minute vessels are found chiefly in fibro-vascular axes radiating from the base of the mesentery, and are surrounded more or less completely by fat storage cells and lymphatic vessels. In both animals as a general rule small arterioles arise near the base of the mesentery, each arteriole splitting into a group of capillaries which are gathered together into one or

[1] The collodion sacs were entirely impermeable to protein and were prepared from a 6 per cent solution of Dupont Parlodion in equal parts of alcohol and ether.

more venules at the opposite extremity of the mesentery. The capillaries of the rat wind in and out between the fat cells, only occasionally presenting clearly visible loops, or more rarely, plexuses in the thinner portions of the mesentery. In the guinea pig mesentery, however, there is less fat and the blood vessels, even to the finest capillaries, are more easily visible.

In these determinations the term artery is used to include the mesenteric artery and its branches with diameters above 40 micra. The vessels termed arterioles measured between 15 and 35 micra, but they were identified by their structure and their relation to the capillary network rather than by their size. Pressures were always taken as close as possible to their final division into capillaries, so that the figures given represent the

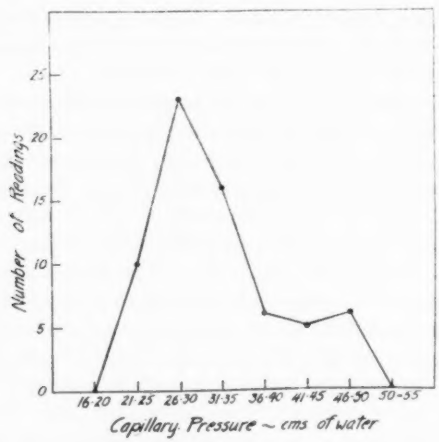

Fig. 1. Chart showing the range and distribution of capillary blood pressure readings in the rat and guinea pig.

pressure at the very end of the arterial tree, immediately before the blood passes into the broader capillary network.

The capillaries measured between 5 and 20 micra, and in two instances 25 micra. The vessels termed venous capillaries were somewhat larger in size, between 20 and 40 micra, with simple endothelial walls and they were usually formed by the union of two or three arteriolar capillaries. They were obviously the finest ramifications of the venous system, but there was no connective tissue coat visible in the living preparation. The venules and veins measured above 70 micra. This classification is mentioned merely for the purpose of identifying definitely the portions of the peripheral vascular system in which the pressure determinations were made. In a description of the blood vessels in human muscle Kernohan,

Anderson and Keith (1929) define as arterioles those arterial branches having diameters between 25 and 100 micra.

b. Range of pressure in the mesenteric capillaries. In a series of measurements in a total of 66 capillaries in the two forms the capillary pressures ranged from 22 to 49 cm. of water. These values are charted in figure 1 to show their relative frequency. Almost two-thirds of the observations lie between 25 and 35 cm. of water, and over one-third between 25 and 30. The most frequently observed single pressure was 30 cm. of water, which was noted in 13 of the 66 observations. The wide variation is probably due in part to the inclusive character of the measurements. They were purposely made to include capillaries with both rapid and slow rates of

TABLE 1

Mean blood pressures in mesenteric vessels of rat (in centimeters of water)

	AORTA	ARTERY	ARTERIOLE	CAPILLARY	VENOUS CAPILLARY	VENULE AND VEIN
Average	97	96	50.5	30	17	14.5
Highest	108	110	52	34	20	15
Lowest	88	70	40	22	15	12
Number of observations	5	6	10	36	10	3

TABLE 2

Mean blood pressures in mesenteric vessels of guinea pig (in centimeters of water)

	AORTA	ARTERY	ARTERIOLE	CAPILLARY	VENOUS CAPILLARY	VENULE AND VEIN
Average	110	106	76	38.5	17	12.5
Highest	115	118	97	49	19.5	14
Lowest	98	90	60	31	13	10
Number of observations	8	4	14	30	20	8

flow in order to obtain a fair average figure. In general the lower pressures were accompanied by slower rates of flow, as in the frog. With an average rate of flow the pressures rarely exceeded 35 cm. of water.

The rapid heart rate prevented the determination of pulse pressure in the capillaries, but the rhythmic movement of the corpuscles when pressure equilibrium was established showed that a slight pulse was present even in the capillary network. Respiratory variations in capillary pressure were present as indicated by slow movements of the corpuscles toward and away from the pipette synchronously with respiration, but the magnitude of these changes was too small to measure accurately.

c. The fall of blood pressure in the mesenteric vessels. The gradient of

pressure fall from artery to vein through the capillary bed is of interest in relation *a*, to the proportion of peripheral resistance which may be located in the capillary network and in the arterioles, and *b*, to the colloid osmotic pressure of the blood. In the frog the fall in blood pressure continued through the capillaries, in agreement with the conclusion of Dale and Richards (1919) that there is no reason to assume an abrupt flattening of blood pressure at the junction of the arterioles and capillaries. This is true in the mammal also as shown by tables 1 and 2 and by figure 3.

A series of 154 determinations of blood pressure in various portions of the mesenteric circulation was made, including 13 determinations of aortic pressure, 10 of mesenteric artery pressure, 24 in the finer arterioles, 66 in the

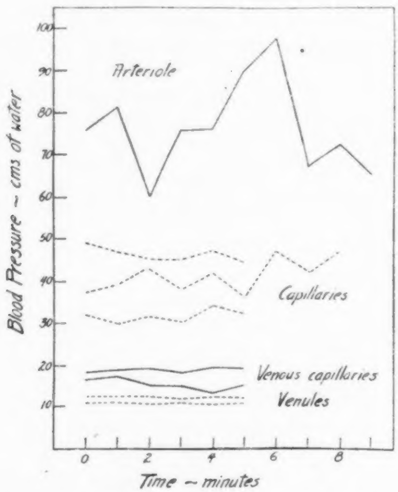

Fig. 2. Chart showing the variations in blood pressure in single arterioles, capillaries, venous capillaries and venules.

arteriolar end of the capillary network, 30 in the venous capillaries and 11 in the venules and veins. The averages for each animal are shown in tables 1 and 2, with the highest and lowest values observed in each group.

The variations in pressure observed in each location when measurements were repeated at one minute intervals are shown in figure 2. The arteriolar pressures varied markedly in successive determinations and were by far the most changeable of the pressures measured, though never falling as low as the highest capillary pressures. The fluctuations were usually restricted to 10 cm. of water, but in a few instances exceeded 35 cm. Capillary pressure also varied from minute to minute, but in most instances only by 5 to 8 cm. of water, rarely over 12 cm. These variations were also some-

what restricted in range, apparently never reaching arteriolar pressure, though at times falling to venous capillary pressure. The blood pressure in the venous capillaries was almost constant, rarely changing over 5 cm. and that in the venules and veins was even more uniform.

The gradients of blood pressure fall in the peripheral vessels of the rat and guinea pig have been superimposed in figure 3 on the curve previously

TABLE 3

The distribution of the fall in blood pressure between the arterioles and the capillary network

	TOTAL FALL IN MEAN BLOOD PRESSURE (ARTERY TO VEIN)	FALL IN MEAN BLOOD PRESSURE			
		Artery to capillary		Capillary to vein	
	cm. water	*cm. water*	*per cent of total*	*cm. water*	*per cent of total*
Frog	23.5	16.5	70	7.0	30
Rat	81.5	66.0	*81	15.5	19
Guinea pig	93.5	67.5	72	26.0	28

TABLE 4

Relation between capillary blood pressure and the osmotic pressure of the plasma proteins

	CAPILLARY PRESSURE	OSMOTIC PRESSURE OF PLASMA PROTEINS	VENOUS CAPILLARY PRESSURE
	cm. water	*cm. water*	*cm. water*
Rat	30.0	25.5 25.5 22.0 25.0 25.0 26.5	17.0
Guinea pig	38.5	22.0 27.5 24.0	17.0
Frog	14.5	11.5* 10.0–11.9†	10.0

* By direct measurement of fluid movement through the capillary wall (Landis, 1927).

† White, 1924.

reported for the frog (Landis, 1926). The fundamental similarity of the three curves is evident. In each instance there is no significant drop in blood pressure in the mesenteric arteries. The first and largest fall occurs in the finer arterioles, with a smaller reduction between the arterioles and the capillaries just beyond. The gradient of pressure fall continues

unbroken through the capillary network, and the curve flattens only in the region between the venous capillaries and the venules.

The percentage distribution of pressure fall between the arterioles and the capillary network has been computed from the data given above. The total fall in pressure through the mesenteric blood vessels was obtained by subtracting average venous pressure from average arterial pressure. The fall of pressure *a*, from the artery to the beginning of the capillary network (namely, capillary pressure); and *b*, from this point to the

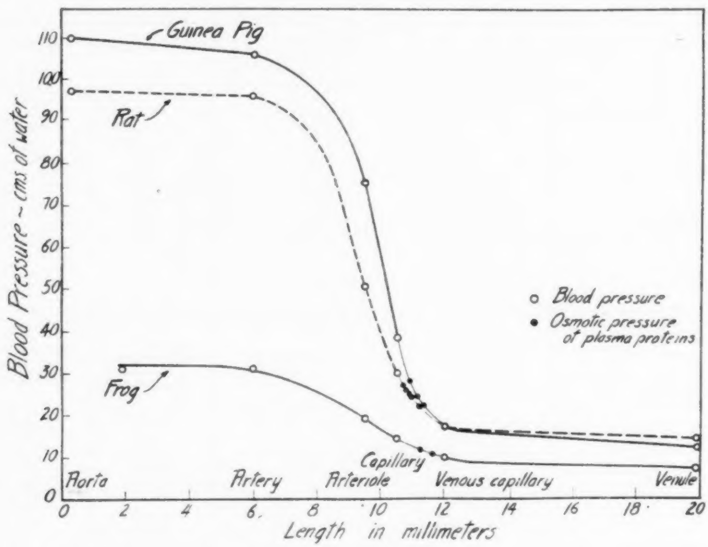

Fig. 3. Chart showing blood pressure fall (circles) in peripheral vessels of the rat, guinea pig and frog. Thin line indicates blood pressure in the capillary network. Dots indicate the osmotic pressure of the plasma proteins.

vein is expressed in table 3 in percentage of the total fall of pressure. The division of the peripheral resistance between the arterioles and capillaries appears to be quantitatively similar in the three forms, in spite of the different level of pressure at which mammalian circulation is maintained.

d. The relation between capillary blood pressure and the osmotic pressure of the plasma proteins. Table 4 shows the results of 8 determinations of the osmotic pressure of the plasma proteins, compared to the blood pressures in the arteriolar and venous portions of the capillary network. In each type of animal the average pressure in the arteriolar portion of the capillary network is higher than the osmotic pressure of the plasma proteins, while the venous capillary pressure is lower.

The pressure relationships in these three forms are shown graphically in figure 3. The mean blood pressure curves have been drawn with a light line to indicate the pressure exerted on the blood as it passes through the capillary network, where the simple endothelial wall permits fluid interchange. The osmotic pressure figures for each animal have been charted on the respective blood pressure gradient curve. The average fall in blood pressure is such that filtration occurs in the arteriolar portion of the capillary network and reabsorption in the venous portion.

These experiments performed in mammals seem to provide additional evidence in favor of the Starling hypothesis of fluid interchange. Apparently the rat and guinea pig differ from the frog in the higher level at which the balance between capillary pressure and the colloid osmotic pressure of the blood is maintained.

Discussion. Capillary blood pressure has not previously been measured directly in the lower mammals. In man, however, Carrier and Rehberg (1922) have made direct determinations by inserting pipettes into the venous capillaries at the base of the finger nail. As mentioned by Kylin (1926) and by Krogh (1929), with obstruction of flow the pressure measured is that of the smaller venules rather than true capillary pressure. Nevertheless certain of the pressures observed by Carrier and Rehberg were lower than the venule pressures found in the rat and guinea pig. In part this may be due to the negative intrathoracic pressure which they mention as a factor in their measurements. In addition the mesenteric veins are a portion of the portal circulation, with a secondary capillary resistance interposed by the hepatic vessels. The venule pressures here reported agree quite well with the portal vein pressures reported by Bayliss and Starling (1894). The slight difference between the two series of observations is no doubt due to the fact that the smaller mesenteric veins investigated in these experiments are peripheral to the portal vein.

Numerous indirect measurements of blood pressure in various sections of the peripheral vessels have been made, but the objections to these have already been considered (Landis, 1926) and the difficulties of interpretation summarized (Krogh, 1929). L. Hill (1928) criticised the direct method here employed on the ground that circulation is impeded by the introduction of the micro-pipette into the observed capillary. This objection is not valid, since in all of these measurements, and in all but five in the frog, a collateral capillary was used as a side tube and the micro-pipette was inserted into this vessel. This allowed circulation to continue in the observed vessel without any possibility of obstruction. In those few experiments in the frog where systolic and diastolic pressures were measured by inserting the micro-pipette directly into the capillary observed, the blood flow was not measurably obstructed. On account of these precautions, directed specifically toward the maintenance of a normal,

unobstructed blood flow during the observation, it is believed that the direct method provides accurate measurements of normal capillary blood pressure.

SUMMARY

The micro-injection method for determination of blood pressure in individual capillaries was used in studying pressure fall in the mesenteric vessels of decerebrate rats and anesthetized guinea pigs. This method permitted the measurement of mean pressure, without disturbance of flow in the observed vessel.

Capillary blood pressures ranged from 22 to 49 cm. of water with two-thirds of the determinations lying between 25 to 35 cm. of water. Average pressure in the arteriolar capillary was 30 cm. in the rat and 38.5 cm. in the guinea pig, while in the venous capillary the average blood pressure was 17 cm. in both forms.

The fall of blood pressure did not cease abruptly at the junction of the arteriole and capillary, but continued to the venous capillary before flattening. The pressure gradients of the rat, guinea pig and frog showed a similar division of peripheral resistance between the arterioles and capillaries.

A balance between average capillary blood pressure and the osmotic pressure of the plasma proteins has been observed in the rat, guinea pig and frog. The mammals, however, differ from the frog in the higher level of pressure at which this balance is maintained.

Average arteriolar capillary blood pressure was above, and average venous capillary pressure was below, the osmotic pressure of the plasma proteins. The findings provide evidence in favor of the Starling hypothesis of fluid interchange in mammals.

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THE RELATION OF PARTICLE SIZE TO MECHANISM OF DYE EXCRETION BY THE KIDNEY[1]

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Early in the course of their study of dye excretion by the kidney investigators noted that marked differences in degree and rate of elimination exist between the various substances which they used to test kidney function. Several theories have been advanced to account for these differences, but one of the most satisfactory and the one perhaps best supported by experimental data is that which holds that the degree of filterability of a dye determines its appearance in the urine. A recent supporter of this theory is Grollman (1925). In his work Grollman finds a close correlation between the elimination of certain dyes in the urine and the results of corrected methods of ultra-filtration through collodion membranes, although other factors such as "binding" of the dye by the body tissues and fluids, for example, may modify the general rule.

We have been interested for some time in the manner of excretion of certain dyes by the perfused frog's kidney (1929, 1929a, 1929b). Briefly, we have presented evidence that phenol red is eliminated chiefly through the glomeruli and that neutral red is in by far the greater part excreted through the tubules. Now it is evident that one may not only compare the elimination of these dyes and others which are excreted in varying degrees by means of the method of perfusion with their filterability, thus testing Grollman's work from a different standpoint, but also may extend the theory to see if the degree of filterability affects in any way the mechanism of their elimination, that is to say, whether the substance is excreted through glomeruli or tubules.

Methods. We have previously described in detail our perfusion method (1929). It is a modification of Barkan, Broemser and Hahn's (1922) and of Höber's technique (1927), the most important change being an increase in the pressures used. The importance of this modification we believe to be fundamental, for it produces a flow of urine of normal proportions and

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council of Pharmacology and Chemistry, American Medical Association.

thus allows an accurate quantitative study to be made of rates of excretion. As we will attempt to show, such data allow a recognition of the relative importance of various processes that is entirely obscured if qualitative relations are alone considered.

In our method of ultra-filtration we have followed the technique of Krueger (in press). An especially designed holder supported a 3 per cent collodion-acetic membrane, and a negative pressure of from 2 to 4 cm. of Hg was applied. Due precaution in making the membranes similar in density was observed and possible variations checked by observations on the rate of water filtration. Due allowance was also made for absorption of the dye by the filters (Grollman, 1925).

In the case of neutral red an ultra-microscopical examination was made of the dye solution under varying conditions to be described later. A Siedentopf slit microscope was employed for this semi-quantitative procedure, using arc illumination and Bausch and Lomb water immersion objectives 7 and 4 mm.

TABLE 1

Filterability of dyes

Concentration 20 mgm. dye in 1000 cc. Locke's solution

	1	2	3	4	5	AVERAGE
Phenol Red	100	98	100	100	100	100
Indigo Carmine	80	85	91	87	92	87
Toluidin Blue	68	53	63	62	51	59
Neutral Red	40	34	36	36	30	35
Trypan Blue	+*	+*	+*	+*	+*	
Brilliant Red	0	0	0	0	0	

* Filtrate pink, no color match with standard possible.

The dyes used were the two whose excretion we have previously studied, phenol red and neutral red and four others whose filterability varies sufficiently to test our hypothesis that filterability may determine the mechanism of excretion. After some trial brilliant red, indigo carmine, toluidin blue and trypan blue were selected.

Experimental. *The filterability of the dyes.* Table 1 shows the filterability of the six dyes. The figures given are a few examples of many experiments. In them a battery of 6 filters, whose membranes had been made at the same time, was run, each filter containing a different dye. Samples of dye were taken at convenient intervals, the filtrate being compared in a Dubosq colorimeter to the original solution which had a concentration of 20 mgm. per 1000 ccm. of Locke's fluid. In every case where dye appeared in the filtrate, there was noted an increasing degree of fil-

TABLE 2

ARTERIAL FLOW	VENOUS FLOW	URINE RATE	CONC. FACTOR* OF DYE	DYE RATE	SUGAR	CONC. FACTOR* OF SALTS
Phenol red (0.02 mgm. per cc.) to tubules						
cc. per hour	*cc. per hour*	*cc. per hour*	*per cent*	*mgm. per hour*		*per cent*
420	300	3.6	69	0.05	0	40
540	200	3.0	150	0.09	0	40
Phenol red to glomeruli						
240	200	2.4	333	0.16	0	40
360	200	4.2	540	0.45	0	40
2 per cent urethane to tubules						
300	150	4.2	400	0.33	±	50
540	200	10.8	500	1.08	+	65
Indigo carmine (0.02 mgm. per cc.) to tubules						
480	300	2.4	100	0.04	0	40
480	300	1.8	120	0.04	0	40
Indigo carmine to glomeruli						
440	500	3.6	110	0.08	0	40
520	480	4.8	158	0.15	0	40
440	320	6.8	300	0.40	0	40
2 per cent urethane to tubules						
440	200	14.0	200	0.56	tr.	50
420	300	24.0	370	1.70	+	65
Toluidin blue (0.008 mgm. per cc. to glomeruli)						
480	600	3.0	25	0.006	0	45
480	600	2.0	25	0.004	0	45
Toluidin blue to tubules						
440	320	2.4	30	0.004	0	45
440	300	5.2	67	0.025	0	50
400	250	5.6	60	0.026	tr.	50
320	280	6.0	85	0.040	+	60
300	250	6.0	80	0.038	+	60
Neutral red (0.0125 mgm. per cc.) to glomeruli						
360	400	5.4	10	0.005	0	40
360	400	7.2	33	0.03	0	40

* Concentration factor $= \dfrac{\text{concentration of dye or salt in urine}}{\text{concentration of dye or salt in perfusion fluid}}$.

TABLE 2—*Concluded*

ARTERIAL FLOW	VENOUS FLOW	URINE RATE	CONC. FACTOR* OF DYE	DYE RATE	SUGAR	CONC. FACTOR* OF SALTS
Neutral red to tubules						
cc. per hour	*cc. per hour*	*cc. per hour*	*per cent*	*mgm. per hour*		*per cent*
400	440	9.0	400	0.45	0	40
400	460	8.4	500	0.52	0	40
540	500	7.8	650	0.63	0	40
2 per cent urethane to tubules						
600	540	18.0	140	0.36	+	55
540	540	21.6	100	0.27	+	70
Trypan blue (0.0125 mgm. per cc.) to tubules						
320	400	0.8	Ft. tr.	–	0	40
400	380	1.8	Ft. tr.	–	0	40
Trypan blue to glomeruli						
560	240	1.6	Tr. pink	–	0	40
480	240	1.6	Tr. pink	–	0	40
400	240	1.8	Tr. pink	–	0	40
2 per cent urethane to glomeruli						
300	240	6.0	Purple	–	Tr.	60
400	240	8.4	100	0.10	+	60
Brilliant red (0.01 mgm. per cc.) to both tubules and glomeruli						
680	320	6.6	0	–	0	40
660	420	7.2	0	–	Tr.	50
2 per cent urethane to glomeruli						
420	300	16.2	Ft. red	–	0	55
480	300	18.0	Ft. red	–	+	60

tration until the membrane was saturated and this final constant figure was taken as the index of filterability.

According to their degree of filterability the dyes may be arranged in the following series of decreasing values: phenol red, indigo carmine, toluidin blue, neutral red, trypan blue and brilliant red. Trypan blue forms an interesting special case whose importance will be apparent later. The filtration separated the two color components of the dye to a certain degree, so that the filtrate was pink in color and no match with the original solution in the colorimeter was therefore possible.

The manner and degree of dye elimination by the kidneys. Similar experiments were made with each of the dyes. In all cases the kidneys were perfused as in our previous experiments, first either by the vein or by the arteries, to test either tubules or glomeruli, and then the method of perfusion reversed. After a certain number of periods of 15 minutes each, the tubules, and in certain cases the glomeruli, were damaged by perfusion with fluid containing 2 per cent urethane in order to check, as in our previous experiments, the effect of damage on the mechanism of excretion. Table 2 illustrates the results of typical experiments.

In the phenol red experiment the same results are found as in our former experiments. A considerably greater amount of dye was excreted when it was supplied to the glomeruli than when it passed to the tubules. Damage to tubules which produced the usual evidence of loss of tubular absorption, that is, a diuresis and glycosuria, caused not a decrease but a definite increase in dye elimination. The significance of this increase and of the high concentration factor accompanying it, we have previously discussed (1929a).

With indigo carmine similar results were obtained. The increase in excretion through the glomeruli over that of the tubules was striking, and as a result of the large diuresis which developed on tubular damage, there was a correspondingly large increase in dye excretion, a result of lack of dye absorption and "anomalous secretion" (1929a).

In the case of toluidin blue a contrast to the results described above was noted. Since this dye resembles in its filterability neutral red much more closely than phenol red, it was suspected that its method of excretion might resemble that of the latter. It was therefore perfused first to the glomeruli and then to the tubules. The excretion through the glomeruli was low, and though there was a definite increase when the dye was supplied to the tubules, the results were not as striking as those we have previously reported with neutral red. It will be noticed that evidence of mild tubular damage developed in the second period following the administration of the dye to the tubules, which increased to a definite glycosuria. Now as we have previously shown, and is illustrated in the next experiment with neutral red, tubular damage lessens the elimination of a substance excreted through the tubule cells, so that the comparatively low excretion of toluidin blue associated with the tubule damage here noted, places it in the same category with neutral red. The tubular damage being the result of the toxicity of the dye itself prevents its tubular excretion to the degree observed with the benign neutral red.

The experiment with neutral red is similar to those previously reported. These was a marked increase in the rate of dye excretion when it was

supplied to the tubules.[2] When the tubule cells were damaged by the urethane, however, there was a sharp drop in the rate of elimination, and this in spite of the fact that the rate of water excretion trebled, a marked contrast to the associated increases of dye and water seen with phenol red and indigo carmine.

Trypan blue, of much less filterability than the dyes so far studied, was observed in the faintest discernible trace when administered to the tubules. When given to the glomeruli there was a slight increase in its elimination, but no comparison with the perfusion fluid could be made as the dye in the urine was faint pink in color. A striking change occurred when the glomeruli were damaged with urethane. The urine became purplish in tinge and finally the same bluish purple color of the original dye, so that a perfect match could be made in the colorimeter. The tubules were also damaged by the arterial administration of urethane, for sugar appeared in the urine.[3] Only faint traces of dye therefore pass through the tubules though a slight excretion of the filterable pink element of trypan blue evidently occurs through the intact glomeruli, and when the glomerular membrane is damaged the dye may pass through unaltered.

Brilliant red, an unfilterable dye, did not appear in the urine when supplied to both glomeruli and tubules simultaneously, provided the glomerular membrane was intact. When the latter was damaged by 2 per cent urethane traces of it appeared in the urine.

In comparing the excretion of these dyes with their filterability a striking analogy is found. In a general way the more filterable the dye, the more readily is it excreted by the kidney. Toluidin blue forms an exception to this rule, since being more filterable than neutral red it is less readily excreted, but an explanation of this anomaly is found in the fact that the dye damages that part of the kidney unit, the tubule, which is responsible for its elimination. Trypan blue, whose excretion is as scanty as its filterability, is also of especial interest, since in both processes the dye is

[2] Our experimental findings with neutral red, as do those of Scheminsky, seem to show that the usual conception of the blood supply to the tubules of the frog's kidney, as given by Cushny for example, must be modified. It would seem that if *any* portion of the tubule is supplied by the anastomoses of the efferent vessel of the glomerulus that this portion must be a different part from that supplied directly by the renal-portal vein. Some region supplied by the renal-portion system can eliminate neutral red, while the region which *may* be supplied by the efferent vessels from the glomeruli cannot. Policard, (Arch. d'Anat. micros., xii, 177, 1910) in fact, states that in the frog the efferent vessel of the glomerulus does not supply segment II of the tubule, but only segments I, III and IV. The broad segment II, the analogue of the proximal convoluted tubule in mammals, is therefore most likely the chief seat of elimination of neutral red.

[3] We shall shortly publish a description of the abnormalities occurring in such glomerular damage during perfusion with more details of the functional and anatomical changes.

separated into its component parts and appears in the urine or filtrate, as the case may be, markedly and similarly altered. Grollman's correlation of filterability and excretion of dyes is therefore observed in the perfused frog's kidney.

A further step in the analysis of the process of excretion may be taken from the findings of our experiments. Those dyes which are readily filterable, phenol red and indigo carmine, pass through into the urine chiefly through the glomeruli. Dyes of a moderate degree of filterability cannot pass through the glomerular membrane readily, but require the activity of the tubule cells for their elimination. Still more colloidal substances, trypan blue, cannot be eliminated by the tubules in the period of time allowed in our experiments to any considerable amount. That portion of this dye, the pink element, which is filterable may pass, however, through the glomerular membrane, and the amount of this passage increases when the membrane is made more permeable by damage, a very striking analogy to the corresponding separation of the dye into its two components by ultrafiltration. The highly colloidal brilliant red can neither pass the glomerular membrane nor can it be handled by the tubule cells.

Filterability would seem therefore to determine not only the degree to which a dye is eliminated but also the mechanism by which it is excreted.

A chance occurrence during the course of one of our experiments lends considerable support to this theory.

During the course of our perfusions the reaction of the Locke's solution is kept constant at a pH of 7.4 to 7.6 by aeration with a proper mixture of CO_2 and O. In a routine experiment on the excretion of neutral red a gradual and marked fall in rate of dye elimination occurred, and accompanying this fall it was noted that, through failure of the aeration system, the Locke's fluid containing the dye had become alkaline. When the reaction of the perfusate was corrected, the rate of dye excretion rose to its former level.

The constancy of this relation of acidity to rate of neutral red elimination was tested and repeatedly confirmed. Figure 1 shows the results of such an experiment. Neutral red in the usual concentration, 12.5 mgm dye in 1000 ccm., was perfused to both glomeruli and tubules in order that the kidney might have an excess of dye. The hydrogen ion concentration of the Locke's solution was regulated by aeration with a proper mixture of CO_2 and O, the neutral red serving as an indicator. During the first period the pH was the normal one of 7.4. The volume of urine was 9.0 cm. per hour, there was no sugar present and the rate of dye excretion was 0.37 mgm. per hour. The acidity of the perfusion fluid was now increased to a pH of 7.0 and maintained at this level for periods 2 and 3. There was an immediate increase in excretion of water and sugar appeared in the urine. In spite of this evidence of tubular insufficiency, however, there was a

marked increase in the rate of elimination of neutral red (1.7 mgm. per hour). In period 4, when the acidity was lowered to a pH of 7.8, a fall in rate of dye excretion resulted. In period 5 an inadvertent rise in H ion concentration due to irregularity in aeration was accompanied by a rise in dye output, which fell promptly when the acidity was lowered again, finally reaching the very low figure of 0.001 mgm. per hour. During these last periods the normal resorptive activity of the tubule had evidently been reëstablished, since the volume of urine was moderate and sugar had disappeared.

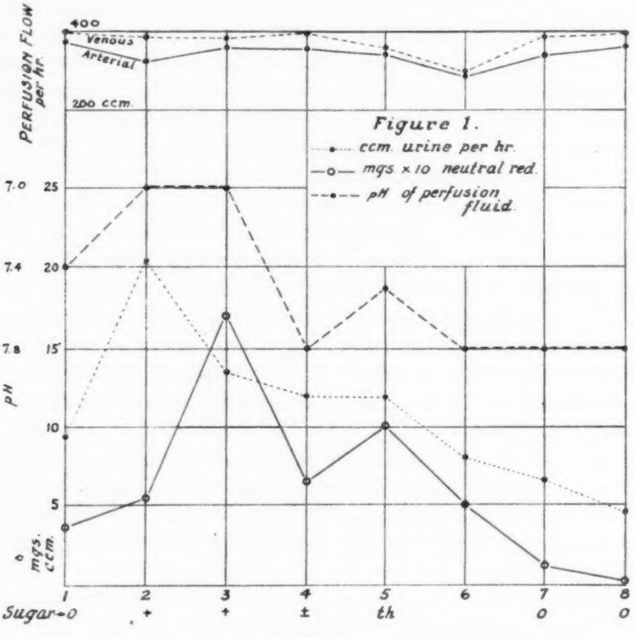


Fig. 1

The results of this experiment are therefore strikingly different from those reported previously, in this paper and our previous publications, for hitherto tubular damage has always been accompanied by a definite decrease in the excretion of neutral red. Moreover in the last few periods of the experiment there was no evidence that the tubule cells were not functioning normally, and one would therefore expect a good output of neutral red. Evidently some other factor had entered into the process of elimination and a clue to this complicating element was found in the appearance of the perfusion fluid after it has stood some time at the low de-

gree of acidity of pH 7.8. In an hour or so the solution became distinctly turbid, and microscopical examination showed it to be filled with small granules and even crystals of dye. It had been thrown from solution by the alkalinity of the fluid.

It has long been known that neutral red is precipitated from solution by OH ions (Teague and Buxton, 1908), and although in our experiments no gross evidence of such an extreme change had occurred during the course of the perfusion, it was entirely possible that an invisible change of similar nature had occurred with the fall of acidity. An increase in size of the dye particles, with a resulting change in filterability, might modify the rate of excretion of the dye.

This possibility was examined by ultrafiltration of the dye in acid and alkaline solutions. The same method of filtration was used as previously

TABLE 3

Filterability of alkaline and acid neutral red: stabile neutral red solution in Locke's fluid

Concentration 8 mgm. in 1000 cc.

	1	2	3	4
pH 7.7	15		25	25
	9	5	25	47
	12	6	27	49
pH 7.0	26	32	66	80
	44	61	70	80
	56	64	62	77
	65			
	64			

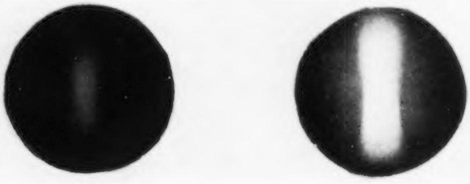
described. Since, however, it was desired to examine the dye solution under varying conditions of acidity but in the absence of gross precipitation a "stabile" solution of neutral red in Locke's fluid was used. It was found that for every degree of acidity there is a corresponding limiting critical concentration, above which point supersaturation and precipitation occur. A solution of neutral red exceeding this concentration was brought to a pH of 7.7 and allowed to stand 48 hours. The precipitated dye was then removed by prolonged and rapid centrifugalization and the supernatant used in the filtration test. Such a solution contained 0.008 mgm. neutral red per ccm. and remained clear tomicroscopical examination indefinitely even at this degree of alkalinity.

In the filtration experiment a series of filters was started with this stabile solution at a pH of 7.7. When a constant rate of dye filtration had occurred, the solutions remaining in the filter reservoirs were made acid to

a pH of 7.0 by a minute drop of n/10 HCL and the filtration continued until a constant figure was again obtained. Table 3 shows the results of several such experiments.

As is evident from the table the acid dye is more filterable than the alkaline. That this increase in filterability is due to decrease in particle size seems likely since a change in charge of the membrane from the addition of the H ions could affect the passage of the dye only slightly if at all, as the H ions do not reverse but only lower the negative charge of a collodion membrane. A further and direct demonstration that the effect of OH ions is to lessen the degree of dispersion of the neutral red and to increase the size of its particles was obtained by examination of the dye solution with a Siedentopf slit ultramicroscope.

A special chamber of soft rubber was devised which was filled with stabile neutral red solution in Locke's fluid at a pH of 7.0. Under proper arc illumination and with water immersion objectives 7 and 4 mm. a faint



A B

Fig. 2. Untouched photograph of A, acid neutral red, pH 6.0 and B, same solution made alkaline, pH 8.0. Siedentopf slit microscope Bausch & Lomb ocular 10, water immersion objectives 7 and 4 mm., 10 seconds' exposure, Orthonon plate.

reddish beam was seen in which minute faint particles were just visible. The solution in the cell was then made alkaline by touching the fluid with a fine glass rod which was covered with a thin film of NOH. Immediately there appeared in the pale reddish beam a few much larger bright yellow specks which gradually increased in number until the whole cone was a dazzling mass of golden particles, easily visible as such. Yet this same fluid when removed from the cell appeared to the eye as clear and transparent as did the acid form. Figure 2 is an untouched photograph taken of the dye under the two conditions of H ion concentration. There can be no doubt, therefore, that there occurs, invisible to ordinary microscopical examination, an increase in the size of the neutral red particles with increases of OH ion in the solution, or a converse decrease in size with increases of H ions, and that these changes are chiefly responsible for the variations in the filterability of the dye under these varying conditions.

The question presents itself now if these alterations may not affect the mechanism of excretion of the neutral red, as well as simply the degree

of its elimination as our previous experiment showed. If this can be demonstrated it will afford an example of a single substance which is excreted by different methods depending on the size of its particles. Figure 3 shows such an experiment.

The general method of perfusion is similar to that of the previous experiments of table 2. The solution of neutral red used, however, was one stabilized at a pH of 7.7 and contained only 0.008 mgm. per ccm. Since the concentration of the perfusion fluid was thus much less than in

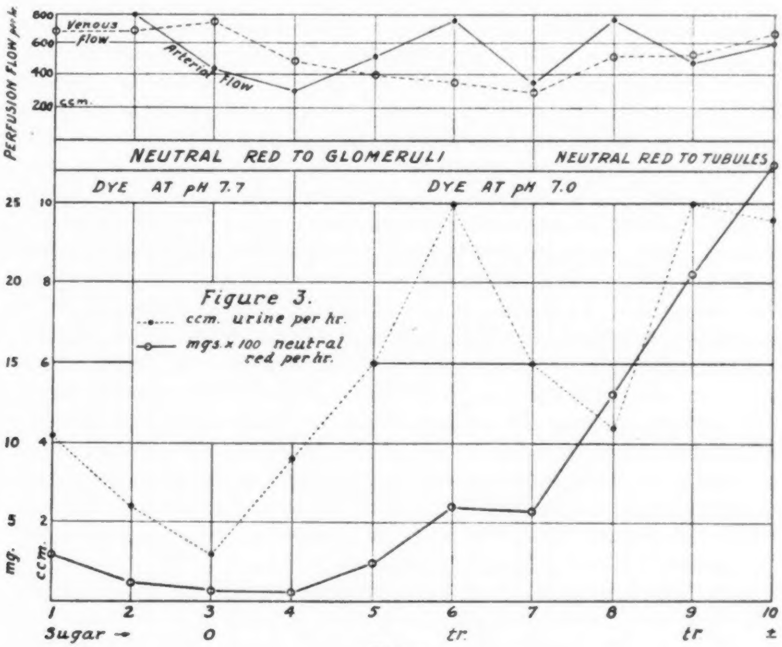


Fig. 3

preceding experiments the amount of dye excreted in the experiment to be described was also less. During the first seven periods the neutral red passed by way of the arteries direct to the glomeruli. In periods 1, 2 and 3 it was quite alkaline, pH 7.7. Only very small amounts appeared in the urine, 0.01 mgm. per hour. The volume of urine was small, evidently due to vascular spasm, as it will be noted that the arterial flow decreased during this period of alkalinity. During periods 4, 5, 6 and 7, the dye still passing only through the arteries to the glomeruli, the solution was maintained at a relatively high degree of acidity, pH 7. As will be noted

there was marked increase in the rate of neutral red excretion, reaching an output six times as great as that of the alkaline dye. At the same time there was evidence of slight tubule damage for a diuresis developed and a trace of sugar appeared in the urine, evidently due to the acidity of perfusion fluid. Irregularities in arterial flow, probably due to the same cause, also affected the volume of urine during these periods. At the end of period 7 the acid neutral red was passed to the tubules, and the marked increase that usually accompanies this form of perfusion promptly developed. The experiment shows therefore that a semi-colloidal substance of considerable particle size, which under normal conditions is excreted to a significant degree only through the tubules, may pass through the glomeruli in considerable amount when its degree of filterability is increased by a reduction in the size of its particles. Glomerular or tubular excretion is determined in the case of neutral red apparently by the purely physical change in the size of the aggregates which are to be excreted.

Discussion. Although it has become impossible to give a complete discussion of the literature of even a restricted phase of kidney function, we can hardly pass to the conclusion of this study without considering the relation our work bears to some of the more recent conflicting studies of dye elimination. In the case of phenol red, for example, we find Hayman and Richards (1926) and Hirschfelder and Bieter (1925) emphasizing the importance of glomerular excretion, while Marshall and Crane (1924) and more recently Höber's pupil Scheminzky (1929) believe it to be eliminated chiefly through the tubules. Our investigations, though agreeing with Scheminzky's in regard to the tubular excretion of neutral red, failed to confirm his findings with phenol red, which in our experiments passed chiefly through the glomeruli. A middle position is occupied by Tamura and his co-workers (1927) who find that most dyes, including phenol red, are excreted by both tubules and glomeruli.

A critical examination of the experimental procedure will show however that in many cases the discrepancies are not so much in the experimental results as in their interpretation. And this is particularly true since in many cases results are expressed only in a roughly semi-quantitative manner. A dye is found in the urine, for example, after one procedure "lighter than pallid methyl blue" and in the contrasting procedure, "a little lighter than pallid methyl blue" or the urine was found "to contain a considerable concentration of pthalein" while in the contrasting part of the experiment, "here also the phthalein was excreted." The interpretation of such results is obviously open to considerable variation.

Another criticism of many of the experiments of the past is the dependence on *concentrations* of excreted substances in the urine as indicative of the degree of function of the kidney. A deeply colored urine if small in volume may of course be the result not of great activity of elimination but the

reverse, and this is particularly true in conjunction with the fact that in many perfusion experiments volumes of hundredths of a cubic centimeter per hour, or "sufficient to reach the distal end of the (ureteral) catheter" in a period of $1\frac{1}{2}$ hours are not infrequently reported. Nor can the "concentration" of a dye like phenol red be estimated in a casual manner by its appearance to the unaided eye under conditions, such as obtained in tissues and body fluids, of varying degree of H ion concentration and consequent color tinge.

In a perfusion experiment, and it is with this method that we are at present only directly concerned, we believe three postulates must be satisfied before any conclusion can be drawn from its results.

First, the function of the kidney must be normally established to such a degree that a normal volume of urine is obtained. Such a volume may be easily recognized from Adolph's work (1927). This will allow, with large frogs, of quantitative measures which are of reasonable dependability. Secondly, the results of the excretion of the dye must be expressed as a *rate* rather than as a concentration if the degree of elimination is to be studied. And finally, comparisons of contrasting procedures must be made in the same animal, with the same kidneys functioning under the same conditions.

Under these restrictions the investigation of the elimination of a series of dyes correlated with their degree of filterability has given a series which may be arranged in the following order.

FILTERABILITY	DYE	GLOMERULAR EXCRETION	TUBULAR EXCRETION
Easily filterable	Phenol red	Marked	Slight
	Indigo carmine	Marked	Slight
Moderately filterable	Toluidin blue	Moderate	Moderate*
	Neutral red	Slight	Marked
Slightly filterable	Trypan blue	Pink element-trace	Slight
Unfilterable	Brilliant red	None	None

* Some tubular damage from dye.

To summarize, those dyes which are easily filterable pass into the urine readily in large amounts through the glomerular membrane, whereas those whose particles are of moderate size and which can pass through the glomerular membrane only with difficulty, require tubular activity for their elimination. Dyes with still larger particles are excreted too slowly to appear in the urine during our experiments or, as other types of experiment have shown, do not appear at all. Certain special cases, such as the pas-

sage of the filterable pink element of trypan blue through the glomerulus, and the varying manner of excretion of neutral red depending on the size of its particles at different degrees of H ion concentration, confirm this hypothesis that the size of the particle determines the mechanism of a dye elimination.

With this hypothesis it is not surprising to find that the more diffusible dyes appear in the urine in a certain amount by passage through the tubules. It would be remarkable indeed if the tubular epithelium presented an impenetrable barrier to the passage of such a diffusible substance as phenol red. It passes through the glomerular membrane, so well adapted for a filtration-like process, with much greater ease and in much greater amount, however; though as Richards and Barnwell (1927) have shown diffusion passage through the tubule is possible, and by our hypothesis and findings, probable.

The relations that our findings bear to those of previous workers may now be briefly summarized. In the case of phenol red there is essential agreement with Hayman and Richards and with Hirschfelder and Bieter that the chief point of excretion is through the glomerulus, and with those of the former in particular, that diffusion processes may occur through the tubule wall. There is a converse disagreement with the views of Marshall and Crane and Höber in regard to the importance of elimination of this dye through the tubules, and we have discussed these points in previous communications (1929a, 1929b).

With neutral red, there is disagreement with Edwards' (1925) (working in Marshall's laboratory) statement that neutral red is not eliminated by the kidney and complete agreement with Scheminzky's (1929) (working in Höber's laboratory), demonstration of its tubular excretion.

There is a general agreement with findings of Tamura and his co-workers (1927), though the significance of this agreement is perhaps not great owing to their presentation of qualitative results only and the resulting ubiquity of the processes of elimination which they therefore find.

Our results were obtained, of course, in kidneys functioning under very special and artificial conditions. They were perfused with a fluid free of colloids, and the presence of such substances, the proteins of the blood plasma, for example, may well affect the degree of dispersions and the size of dye aggregates which result from "binding." These aggregates of dye-plasma may be quite different in size from those studied under the simple conditions of our perfusions yet their size may still be a determining factor in the mechanism of their excretion. Differences that may be observed in the excretion of a dye in vivo will not therefore of necessity invalidate the results of our experiment or our hypothesis. And since our results cannot be transferred in toto to the living animal, we have endeavored with a certain success to test the applicability of our hypothesis in the case

of the contrasting mechanisms of neutral red and phenol red excretion in mammals (1929b).

CONCLUSIONS

1. A correlation of the degree of filterability of a series of dyes with the mechanism of their elimination, whether glomerular or tubular, suggests that the mechanism of their excretion is determined by the size of the dye particle.

2. Easily filterable dyes, of small particle size, pass readily through the glomerular membrane; those of moderate particle size require tubular activity for their elimination, and those whose particles are of large size cannot be excreted.

3. The special cases of trypan blue and neutral red elimination confirm this hypothesis. With the latter the decrease in particle size which accompanies increased glomerular excretion under certain conditions can be objectively seen in the ultramicroscope.

4. The relation of these findings to conflicting recent literature is discussed.

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